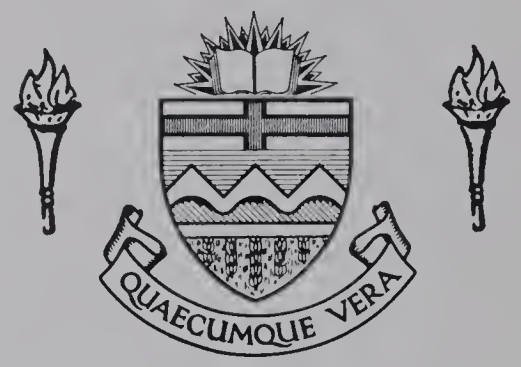

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Food Poisoning Potential of Vacuum Packaged Cooked Ham

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Food Poisoning Potential of Vacuum Packaged Cooked Ham submitted by James E. Steele in partial fulfilment of the requirements for the degree of Master of Science in Foods.

DEDICATION

This is dedicated to my dear father who passed away before this thesis could be finished.

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I thank Dr. M.E.Stiles, my Supervisor, for his help, direction and encouragement given to me during this study. Working with Dr. Stiles was a very rewarding learning experience.

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I. Introduction

> "The present generation is extremely partial to cooked meats which require no preparation before serving and which make such excellent sandwich fillings" (Gerrard, 1960).

However, it was the sandwich fillings which Longree et al.(1959) implicated as the most hazardous component of sandwiches, if they were exposed to storage abuse. Since large numbers of sandwiches, both commercially or otherwise prepared, are consumed daily in North America (Adame et al., 1960; Khan and McCaskey, 1973), it is possible that sandwiches could be vehicles of food poisoning (Anon., 1952). Despite the potential for food poisoning from sandwiches, they have rarely been involved in food poisoning outbreaks (Adame et al., 1960; McCroan et al., 1964).

In this study, vacuum packaged sliced, cooked ham was used as the sandwich filling. Sliced ham, a product of modern, sophisticated processing methods, is an integral meat produced by tumbling/massaging procedures (Anon., 1974; Crittenden, 1974). An integral meat, according to Karmas (1976), consists of chunks of meat bonded together by the proteinaceous exudate which develops during the tumbling/massaging procedures.

The microflora of this product and other cured meats are reported to be heterogenous initially, with subsequent predomination by the lactic acid bacteria (Alm et al., 1961; Kempton and Bobier, 1970; Kitchell and Shaw, 1973; Mol et

al., 1971; Surkiewicz et al., 1977). A low incidence of enteropathogenic bacteria has also been reported (Paradis and Stiles, 1978a; Surkiewicz et al., 1977).

In this study, the bacterial quality of vacuum packaged sliced ham was first assessed by conducting a survey of the product from six federally inspected manufacturing plants in Edmonton. The normal saprophytic flora was determined and the presence of potential pathogens and indicator organisms was monitored. Subsequent to this survey study, sliced ham from two different manufacturers, was obtained directly from the plants and used in inoculation experiments. Ham slices were inoculated with enteropathogenic bacteria and made into sandwiches. The changes in pathogen and saprophyte numbers were monitored under conditions which represented severe abuse, storage at room temperature and under refrigeration.

The data are presented in this thesis in the form of two papers intended for publication in the Journal of Food Protection (Chapters 3 and 4). These papers are preceeded by a review of the literature pertinent to this area of study (Chapter 2). Some general conclusions are included in Chapter 5, and the references pertaining to all parts of the study are given at the end of the thesis.

II. Literature Review

Processed cured meats are frequently purchased because of their convenience, variety and universal availability (Kramlich et al., 1973). These meats are ideal for use in sandwiches, but according to Longree et al. (1959), they represent a food poisoning hazard. As a result, the literature on the microbiology of sliced, cooked ham and related products has been reviewed, with special reference to methods of production and food poisoning hazards.

1. Ham Preparation and Production

Traditionally, ham preparation involved initial trimming of excess fat or rind and ageing of the leg or gammon (Jensen, 1949). The leg or gammon was then cured. Curing time was usually 60 to 80 days because of the processes involved in dry or pickle/brine cure. Dry cure involved mixing the dry ingredients and then hand rubbing the mixture onto the ham (Jensen, 1949). Pickle cure utilized the curing ingredients in solution as a brine. The ham was usually immersed in this brine and allowed to mature for 8 to 10 weeks at refrigeration temperatures (Jensen, 1949). Subsequently, curing methods were modified. In the modern method, the pickle or brine cure is injected by needle(s) directly into the ham musculature, or via the arterial system of the ham, and the ham is then immersed in

a cover pickle for completion of curing (Fields and Dunker, 1952; Halliday, 1967; Jensen, 1949). With the development of injection methods, production time has been decreased to 10 - 20 days (Fields and Dunker, 1952; Halliday, 1967; Jensen, 1949). According to Halliday (1967), half of the remaining production time involves curing the injected ham in the cover pickle, while the other half is used to mature the ham. Ripening of hams can be speeded up by raising the curing temperature (Anon., 1974).

After curing, the ham is washed and dried before cooking/smoking (Fields and Dunker, 1952; Jensen, 1949). Cooking and/or smoking is done until an internal temperature between 61 to 74 C is reached (Fields and Dunker, 1952; Jensen, 1949), after which the hams are chilled to an internal temperature of 4 to 10 C (Fields and Dunker, 1952), and stored at refrigeration temperatures.

The problems encountered with traditional ham manufacture were heavy cooking losses, and the labour needed for their preparation. Furthermore, these hams did not provide the convenience required for the Fast Food era. The slicing of these hams presented particular problems (Karmas, 1976). Skilled carvers were needed, and even then, the uniformity of slicing was not ensured (Karmas 1976). Production methods were again modified, and the concept of an integral meat was developed. According to Karmas (1976), an integral meat consists of small chunks of raw meat, of relatively low value, joined together into larger pieces by

the natural cement-like protein exudate which is produced on the surface of the meat by mechanical work and compression. Integral meats solved the problems associated with traditional meats, while retaining the same texture and high quality of intact meats. The principal advantages are reduced cost of production and utilization of cheaper cuts (Karmas 1976).

Ham production has adopted the new technology of tumbling and massaging (Karmas, 1976; Schmidt, 1977). The first step involves injection of the green ham material with a brine solution (preferably into the musculature) (Michels et al., 1971), to about 25 - 30% of the meat weight (Crittenden, 1974). The injected chunks are then placed in stainless steel vats which are either simple cylinders, the inside surfaces of which are fitted with vanes, which rotated about a central axis (Crittenden 1974; Schmidt, 1977; Woolen 1971), or four-sided or round containers in which there is a vertical shaft with several horizontal arms (Crittenden, 1974; Michels et al., 1971; Schmidt, 1977; Weiss, 1974). The former process, tumbling, utilizes impact energy, while massaging utilizes frictional energy (Weiss, 1974). Both processes result in even cure distribution, thorough mixing of the meat pieces, and the development of a proteinaceous exudate needed for the binding of the pieces (Schmidt, 1977). Massaging and tumbling are both intermittent processes and a good product is obtained if there is stirring or tumbling between 10 - 20 min of each

hour during the total curing period of 18 - 24 h (Michels et al., 1971, Anon., 1977; Schmidt, 1977).

Tumbling/massaging are reported to have many beneficial results besides the primary effect of enabling pieces of meat to be bonded together (Weiss, 1974; Woolen, 1971). According to Crittenden (1974), the first and most important advantage is that tumbling/massaging allows the breakdown of the fibrous structure. This improves the texture of the final product (Anon., 1977). Secondly, Crittenden (1974) stated that these processes increase the activity of the salt and polyphosphate ingredients of the cure, allowing quicker protein (actin/myosin) solubilization and thus better bonding and water retention. The main effects of the polyphosphate are to elevate the pH of the meat and to prevent the development of oxidative off-flavours and off-odours, as well as preverting discoloration by sequestering the alkaline-earth and heavy metal ions (Wierbicki et al., 1976).

The tumbled/massaged product is then potted in stainless steel moulds, under vacuum, since this excludes air pockets, and ensures product uniformity from slice to slice (Anon., 1974). Once moulded, cooking is done. Formed hams are generally cooked to an internal temperature of 68 C, with the cooking cabinet temperature set between 76 - 82 C (Anon., 1974; Crittenden, 1974). Hams cooked in this way are only pasteurized and any subsequent handling and storage has to be done with care and at refrigerated temperatures

(Crittenden, 1974).

The cooked hams are cooled, and stored at 0 C for 48 h (Anon 1974), after which time they are sliced and vacuum-packaged in controlled portions (Anon, 1974). Chopped ham utilizes the same processes. However, the processes used for the latter product are slightly different (Anon. 1974). Ham material is chopped into small particles and suspended in a heat stabilized emulsion (Anon., 1974). This product represents a hybrid of a true emulsion and an integral meat, while retaining the characteristics of the latter. Other processed meats fall into the category of emulsion meat products e.g. sausages and luncheon meats, in which the raw materials are ground before processing.

2. Microflora of Ham and Related Products

The microflora of these cured meat products can be differentiated into the normal saprophytic flora, the indicator organisms and the potentially pathogenic flora. The presence of these organisms can be related to the overall processing techniques and methods employed.

A. The Normal Saprophytic Flora.

During ham manufacture a certain number of saprophytic organisms are expected to survive on the product, and in the case of the lactic acid bacteria, can be considered desirable. Sources and types of bacteria on hams and other cured meats have been extensively studied.

Injected brines are virtually sterile (Halliday, 1967), whereas conventional, curing brines have a varied flora. Deibel and Niven (1958) reported that motile homofermentative lactobacilli are the most common organisms found in ham curing brines, and on the surface of cured, unprocessed hams. They also reported that prompt thermal processing in the smoke house generally eliminates large numbers of lactobacilli.

Salt tolerant organisms predominate the flora of country style hams (Graham and Blumer, 1971). Lactobacilli dominate the butt region, and coagulase negative staphylococci the fore leg region of control hams (Bartholomew and Blumer, 1977). Lancefield group D streptococci, Enterobacteriaceae, Staphylococcus epidermidis, micrococci and Proteus have also been isolated from the microflora of country style hams (Graham and Blumer, 1971; Bartholomew and Blumer, 1977). In addition, Bacillus was reported to be the most common genus in these conventionally processed meats, because of its wide distribution and resistance to thermal processing (Riemann, 1963). However, Bartholomew and Blumer (1977) noted that if a lactic starter is used, these lactic organisms predominate the flora of country style hams.

Langlois and Kemp (1974) reported that in hams stored for 2 -6 days prior to curing, lactobacilli decrease with increase in storage time and temperature. In dry-cured hams, Streptococcus counts are low initially, decrease during

processing (salt equalization at 10 C for 4 weeks, then smoked), and are virtually absent after 1 month ageing at 24 C (Johnson et al., 1975). They stated that B. cereus follows the same pattern.

During ageing of Italian hams, the micrococci and coryneform bacteria are at relatively low levels in lean hams (16 x 10^4/g and 5 x 10^4/g, respectively) after 60 days at 2 C, while on fat hams the microbial counts are much higher (6.2 x 10^6/g for micrococci, and 8.4 x 10^6/g for coryneforms) (Giolitti et al., 1971). Giolitti et al. (1971) also found small numbers (50/g of meat) of Lancefield group D streptococci and lactobacilli in these Italian hams.

An examination of commercially cured hams prepared by four typical methods showed that the majority of bacteria are non-pathogenic micrococci (Dunker et al., 1953). Miller (1960) reported that the microflora of slices of cooked ham obtained from retail stores is dominated by the genus Microbacterium, which is also associated with the souring of the product. Less frequently, lactobacilli, streptococci and pseudomonads are found (Miller, 1960). Lechowich (1971) stated that a species of Microbacterium, together with a Micrococcus, are the predominant organisms responsible for the surface slime on cured hams.

The hot processing techniques used by some manufacturers in the rapid processing of ham does not influence the microflora of the final product. Cornish and Mandigo (1974) indicated that there are no qualitative

differences between the microflora of accelerated processed or conventionally cured ham.

Heat treatment in ham production has an important effect on the microflora of conventional and formed hams. It was found that cooking is sufficient to eliminate the lactic acid bacteria, but processed products become recontaminated during slicing and packaging (Kempton and Bobier, 1970). However, reports indicated that lactic acid and other bacteria can withstand heat processing. For example, Heiszler et al. (1972) reported that for frankfurters, the curing agents, smoking and heat treatment combine to select gram positive organisms, including Micrococcus, Sarcina, Lactobacillus, Microbacterium and Bacillus. It was also stated that in cured meats lactobacilli grow on the surface of the meat as contaminants after heat treatment, or in the interior of the product if they are heat resistant and have survived heating (Sharpe, 1962). Kitchell and Shaw (1973) cited a report in which lactic acid bacteria, in particular the atypical streptobacteria, were shown to survive the scalding and singeing treatments during carcass dressing. Lactic acid bacteria, also Bacillus spp., micrococci and Corynebacterium spp., survive the post-packaging pasteurization applied to packages of baconburger (Gardner, 1968). As a consequence, such cured, packaged meats are stated to be non-sterile products (Richardson, 1973).

The microbiology of ham and related products is also affected by the final manufacturing processes i.e.,

handling, slicing and packaging (Lechowich, 1971). Allen and Foster (1960) stated that the current practice of slicing sausages for consumer packaging has introduced the opportunity for additional contamination. In fact, such slicing and packaging methods would permit the contamination of freshly cut surfaces of cured meat products (Kempton and Bobier, 1970), with lactic acid bacteria e.g. micrococci, lactobacilli and microbacteria (Lechowich, 1971). In addition, Gilbert (1969) reported that the use of contaminated slicing machines and washing cloths results in re- and cross-contamination of the same and other products, respectively, either directly from machines or indirectly from the cloths used in sanitary maintenance of these machines.

The advent of vacuum packaging, used mainly to retail portions of formed hams and other cured meats (Anon., 1974), introduces another major factor affecting the microflora of these products. According to Baran et al. (1970), doubt remains as to the effect of vacuum packaging on microorganisms on cured meats. However, Cavett (1962) and Ingram (1962) both stated that vacuum packaging inhibits or eliminates various organisms. Further, it was reported that vacuum packaging does not hinder the growth or activity of microorganisms normally present on bacon or introduced during processing (Ulrich and Halvorson, 1964). Vacuum packaging (Alm et al., 1961), together with other intrinsic factors (Richardson, 1973), can bring about qualitative

changes or determine the bacterial types that develop on cured meats. For instance, vacuum packaging was reported to lead to the selection of carbon dioxide-resistant lactobacilli and closely related organisms (Reuter, 1973; Riemann et al., 1972; Sharpe, 1962). However, organisms would not be selected unless the meat product was contaminated before packaging (Ingram, 1962).

In Wiltshire bacon, micrococci initially predominate the microflora because of their ability to reduce nitrate to nitrite (Ingram and Dainty, 1971), and to grow in high salt concentrations ≥5% NaCl (Ingram and Kitchell, 1967). As the environment in the closed ecological system changes, enterococci succeed (Cavett, 1962). Ultimately, lactic acid bacteria predominate the microflora (Cavett, 1962; Frazier, 1967; Ingram, 1962). In fact, Mol et al. (1971) reported that after vacuum packaging, 10 - 15% of the microflora of bacon consists of lactic acid bacteria, and that after 1 week at 8 C, the lactic acid bacteria develop to 25 - 30% of the total flora. Finally, the lactobacilli are responsible for the sour spoilage of vacuum packed bacon held at 20 C (Cavett, 1962). Frazier (1967) stated that micrococci and faecal streptococci are also capable of growth in vacuum packaged bacon, especially if the wrapper is somewhat permeable to oxygen. Coagulase negative S. aureus would grow on vacuum packaged bacon at 37 C, but a Lactobacillus-Micrococcus mixture grows at 20 C (Gardner and Kitchell, 1973). In addition, Gardner and Kitchell (1973)

reported that lactobacilli, micrococci and yeasts are frequently found in the spoilage flora of vacuum packaged bacon. In vacuum packaged baconburgers, lactic acid bacteria are dominant, with 88% of the flora being atypical streptobacteria after 3 weeks storage at 4 C (Gardner, 1968).

With respect to the microflora of vacuum packaged, cooked, cured meat products held under refrigeration, different views have been presented of which organisms predominated. Initially, commercially produced, processed meats possess a heterogeneous microflora which is dependent on the product in question, and the basic microflora of the place of manufacture (Alm et al., 1961). The lactic acid bacteria (Kempton and Bobier, 1971), in particular the lactobacilli (Allen and Foster, 1960; Alm et al., 1961; Reuter, 1973; Sharpe, 1962), and the unclassified streptobacteria (Mol et al., 1971), were reported to be the predominant microflora of vacuum packaged, cooked, cured meat products. Shank and Lundquist (1963) pinpointed streptococci as the the major microorganism in the microflora of vacuum packaged, ready-to-eat table meats. A mixture of lactobacilli, Vibrio, M. thermosphactum, and Enterobacteriaceae are frequently found in the spoilage flora of vacuum packaged, cooked meats (Gardner and Kitchell, 1973). Micrococci were also reported to be predominant in some of these products (Brooks and Henrickson, 1956; Miller, 1960; Mol et al, 1971), however,

they seem to be restricted more to the sausage type meats (Frazier, 1967; Palumbo et al., 1974).

The total bacterial population of vacuum packaged, cooked, cured meats depends on the age of the product. This varies from a few hundred to several hundred million organisms per gram of product (Warnecke et al., 1966). Surkiewicz et al. (1977), in their survey of vacuum packaged ham, reported that initial counts were of the order of 2 x 10^3/g or less. Another report indicated that the initial population on vacuum packaged, sliced ham was $\leq 10^3$/g (Shank and Lundquist, 1963). After 28 days, a maximum total population of $\geq 10^8$/g was attained in the product (Shank and Lundquist, 1963).

Low counts ($\leq 10^3$/g) were also obtained on various other vacuum packaged, cured, cooked, meat products (Allen and Foster, 1960; Steinke and Foster, 1951). Warnecke et al. (1966) found that the counts on vacuum packaged bologna varied from 2 x 10^3/g after 1 day to 10^5/g after 10 days, and 10^7/g after 20 days storage at 9 C. Kempton and Bobier (1970) found that for a variety of vacuum packaged, meat products the maximum total count of 10^8/g was attained after 3 - 4 weeks storage at refrigerated temperatures. A maximum total population of 10^8/g was found after 40 days storage at 7.2 C (Allen and Foster, 1960), and was sometimes attained only after 46 - 60 days of storage (Hill et al., 1976). Alm et al. (1961) also reported maximum stationary populations of 10^8/g in vacuum packaged meat.

B. Pathogenic Microorganisms.

The presence of pathogens in vacuum packaged, sliced, cooked ham can result from any or a combination of all of the following causes suggested by Bryan (1974):

(a) contaminated raw materials;

(b) inadequate processing to kill pathogens, or inherent heat resistant strains of pathogens;

(c) foods could become contaminated during post-processing operations (slicing and packaging);

(d) environmental conditions could permit bacteria to multiply to such an extent that they attained numbers sufficient to cause food poisoning.

Even with these possibilities, the incidence of pathogens in processed, cured meat products is generally low. Bryan (1974) stated that bacteria of public health concern are not normally associated with dry cured ham, as evidenced by the lack of food poisoning outbreaks involving this product in the last 14 years. Bartholomew and Blumer (1977) indicated that the small numbers of coagulase positive S. aureus found in the hams surveyed could not cause an immediate food poisoning outbreak. Food poisoning S. aureus die rapidly in plain curing pickle, but survive when soluble meat proteins are present (Lechowich et al., 1956). Lechowich et al. (1956) also reported that when food poisoning S. aureus are injected into hams with the pickle solution, S. aureus survive the normal curing processes until smoking (58.3 C). Graham and Blumer (1971) indicated that aged, dry-cured hams

does not contain coagulase positive S. aureus, E. coli, or Salmonella. However, E. coli (Graham and Blumer, 1971), and low counts of Salmonella (Johnson et al., 1975) have been found in fresh hams. Langlois and Kemp (1974) reported that laboratory-produced, dry-cured hams were free of C. perfringens. Barbe et al. (1966) isolated clostridia from only 4 of 38 ham samples, and it was suggested that the low numbers of Clostridium spores in cured, cooked meats were probably spores that did not germinate during processing (Riemann, 1963).

No cases of food poisoning have been associated with canned Parma ham (sliced, salt-cured but not cooked before canning), although C. welchii (perfringens) type A was isolated (Cragg and Andrews, 1973). Also, no Salmonella or C. botulinum were found in this canned ham product (Cragg and Andrews, 1973).

S. aureus was reported to occur and survive in bacon curing brines and on vacuum packaged bacon (Eddy and Ingram, 1962). Hodge (1960) stated that S. aureus is an ubiquitous organism, and that its occurrence on such handled foods can not be avoided. In addition, Riemann et al. (1972) reported that S. aureus is a naturally occurring contaminant in semi-preserved meat products and that most meat products do not have the brine concentrations required to completely inhibit this organism i.e. 10% sodium chloride. Contrary to the last report, Cavett (1962) stated that coagulase positive S. aureus does not multiply on normal vacuum

packaged bacon (5 - 7% NaCl), although they are occasionally isolated. Lechowich (1971) stated that food poisoning S. aureus multiply to extremely high populations in or on meat products, in particular ham. However, Jay (1962) failed to isolate coagulase positive S. aureus from bologna, salami and weiners.

E. coli, S. aureus and salmonellae were not isolated from any samples taken during the slicing, packaging and refrigerated storage of imported canned ham in the United States (Surkiewicz et al., 1977). However, an examination of cooked, unsliced, ready-to-eat meats (not vacuum packaged) as offered for sale in some retail premises in Ireland, showed that 80% of the samples were E. coli type I positive, 47% had coagulase positive S. aureus, and 21 of 45 samples contained Clostridium spp. (Dempster et al., 1973). Members of the family Enterobacteriaceae were found in vacuum packaged, cooked, cured meats (Gardner and Kitchell, 1973), and E. coli were reported at levels of 10^3/g when packages of cooked, cured meat products were exposed to abusive storage conditions (Hughes, 1970).

Salmonellae are often found in raw meats used for manufacturing processed meat products (Weissman and Carpenter, 1969), and these serotypes can become part of the indigenous microflora of the food processing environment (Tompkin, 1976). However, Davidson and Webb (1973) stated that the available data indicate that vacuum packaged, ready-to-eat, cured meats have seldom been associated with

salmonellosis. In addition, Surkiewicz et al. (1977) failed to isolate Salmonella from vacuum packaged, sliced ham, and Angelotti et al. (1961b) failed to do so from any cured meat products. Palumbo et al. (1974) stated that salmonellae do not survive the heat processing given these products. According to Lechowich (1971), danger of salmonellosis from meat and meat products results from meats recontaminated after processing.

Meat and meat products were reported to be the major vehicles of food-borne B. cereus poisoning (Geopfert et al., 1972). Goepfert et al. (1972) reported that a study done in Russia showed that 13.6% of canned foods and 7.7% of sausage products contained B. cereus. Another Russian study showed that 52.2% of 431 meat and vegetable products contained B. cereus at levels of $\leq 10^2$/g.

Riemann et al. (1972) stated that C. perfringens is seldom involved in food poisoning caused by semi-preserved meat. However, Lechowich (1971) stated that 4.7% of all sliced, luncheon meat products contained C. perfringens. In addition, Strong et al. (1963) reported that 20 of 122 samples of meat, poultry and fish had C. perfringens counts in the range of 10^1 - 10^3 organisms/g.

Insalata et al. (1969) isolated C. botulinum type B spores from only 1 of 400 vacuum packaged meat products examined. However, C. botulinum type B toxin was demonstrated (using an enrichment technique) in 1 of 73 samples of luncheon meat from the California area, but the

organism was not found (Taclindo *et al.*, 1967). In spite of this, botulism is not considered to be a major problem in vacuum packaged fresh and cured meats stored below 10 C (Lechowich, 1971). Manufacturing procedures have been developed to destroy cells and spores with a sufficient margin of safety to make the probability of any survivors extremely remote.

Generally, pathogens develop well if given the right conditions as well as a compatible substrate. For instance, salmonellae and clostridia do not grow well in cured meat products, whereas *S. aureus* do grow well because of their salt tolerance. In fact, it was suggested by Hughes (1970) that *S. aureus* is the real health hazard in vacuum packed meats because the organism is salt tolerant, is a facultative aerobe, and thus can grow readily with low oxygen concentrations.

C. Indicator Organisms.

The routine examination of foods for pathogenic microorganisms and their toxic products is considered impractical in most laboratories (ICMSF, 1978). However, if epidemiological evidence suggests the occurrence of a specific agent in a particular type of food, routine tests for that pathogen or toxin are necessary (ICMSF, 1978).

Methods for the detection of food-borne diseases can be unreliable, especially when the pathogens are expected to be unevenly distributed in foods heavily contaminated by other

organisms (ICMSF, 1978). As a result, analysis of these cooked, cured meats for indicator organisms, in particular faecal indicators, provides the information required to evaluate and draw reliable conclusions about their safety with respect to the possible presence/absence of pathogens. E. coli, coliforms and faecal coliforms have been used traditionally as indicators of contamination in food (Adame et al., 1960; Buttiaux and Mossel, 1961; Thatcher and Clark, 1968). E. coli, in particular, is very numerous in faeces, but it is the least resistant to the extra-enteral environment (Buttiaux and Mossel, 1961).

Although group D streptococci are more resistant than E. coli to the extra-enteral environment, they are less numerous in faeces (Buttiaux and Mossel, 1961; Thatcher and Clark, 1968), and thus have been less frequently used as indicators (Gardner and Kitchell, 1973; Hall et al., 1967; Mol et al., 1971; Oblinger, 1975; Thatcher and Clark, 1968).

E. coli and Lancefield group D streptococci were reported to be good indicators (Buttiaux and Mossel, 1961). However, correlations between coliforms, faecal coliforms and group D streptococci were poor (Buttiaux and Mossel, 1961; Thatcher and Clark, 1968), and it was suggested that group D streptococci and the genus Enterobacteriaceae rather than E. coli alone, be used to obtain more reliable information (Buttiaux and Mossel, 1961).

Some other organisms may occasionally be employed as indicators of poor manufacturing and handling conditions in

vacuum packaged, cured, meat products. Gardner (1968) suggested that the lactic count could be used as an indicator of inadequate refrigeration. Total aerobic counts have also been used occasionally as indicator counts in non-fermented foods to indicate abusive storage, inadequate heat processing, unsatisfactory sanitation, and contaminated raw materials (Thatcher and Clark, 1968). However, Bartl (1973) suggested that the total aerobic count should not be used as an indicator in vacuum packaged foods and refrigerated meats, since the result could easily be misconstrued or misinterpreted.

To interpret results for indicator organisms in vacuum packaged, cured meats, many factors should be considered:

(a) an indicator organism should occur in the intestine as well as in the faeces so as to represent some association with enteropathogens;
(b) in addition, an indicator should be readily detected and be resistant to the extra-enteral environment;
(c) actual faecal contamination is very small;
(d) whether or not the cured product is a cooked, ready-to-eat meat;
(e) the presence of group D streptococci has to be evaluated carefully since in vacuum packaged cured, meat products, they can represent a characteristic part of the normal flora;
(f) finally, and contrary to some of the preceeding points, it should be realized that high indicator counts suggest

exposure to conditions that might introduce pathogens and allow their growth (Buttiaux and Mossel, 1961; Thatcher and Clark, 1968).

With respect to (c), Buttiaux and Mossel (1961) indicated that direct faecal contamination result in transfer of less than 100 Enterobacteriaceae, and less than 10 group D streptococci per gram when the faecal transfer rate is 25 mg/10 kg of food. As a result, high indicator counts suggest that bacterial multiplication occurs during processing and/or storage of the product, rather than from high levels of contamination (Buttiaux and Mossel, 1961; Thatcher and Clark, 1968).

With this in mind, the incidence of indicator organisms in ham and related cured, meat products is reviewed. Bartholomew and Blumer (1977) isolated Lancefield group D streptococci and Enterobacteriaceae from country style hams. In dry-cured hams, group D streptococci are also present in low numbers, but E. coli and Enterobacteriaceae are only found on the fresh hams, not on cured, aged product (Graham and Blumer, 1971). Langlois and Kemp (1974) indicated that numbers of coliforms decline in dry-cured hams after curing and salt equalization, and remain at a very low level for 3 months during ageing and curing.

Giolitti et al. (1971) indicated that low numbers (50/g meat) of coliforms and group D streptococci have been isolated from raw, Italian-type hams. In a survey, low numbers of group D streptococci have been isolated from 7 of

23 tins of canned Parma ham (Cragg and Andrews, 1973), and in self-service, packaged (no vacuum), sliced ham, there was also an infrequent occurrence of these organisms (Miller, 1960). Cavett (1962) indicated that although group D streptococci increased to about 50% of the flora after 9 days storage in aged bacon, they are characteristic of the flora on this vacuum packaged product.

In 1971, the examination of 45 samples of unsliced, cooked, ready-to-eat meats on sale in 15 retail premises in Ireland, showed that 80% were E. coli positive (Dempster et al., 1973). However, in vacuum packaged sliced ham prepared in the United States, no E. coli were found and only 3 of 180 samples surveyed were coliform positive (Surkiewicz et al., 1977). Although vacuum packaging of these cured meat products inhibited the genus Escherichia (Shank and Lundquist, 1963), other members of the Enterobacteriaceae were still isolated (Gardner and Kitchell, 1973).

S. faecalis, S. durans and S. lactis were isolated from vacuum packaged, sliced, cooked meat products, but group D and Q antigens were not detected (Mol et al., 1971). Kempton and Bobier (1970) stated that faecal streptococci are probably not able to multiply at the more restricted temperatures under which these luncheon meats are stored.

To conclude, it could be stated that the counts of all classical indicators in cured, meat products, vacuum packaged or not, are generally low. In addition, the presence of inhibitors probably reduces the possibility of

these organisms developing even though conditions may seem right for their growth.

3. Factors Affecting Survival and Growth of Saprophytes and Pathogens in Vacuum Packaged Ham and Related Products.

Meat provides an excellent growth medium for a wide spectrum of bacteria (Lechowich, 1971). Cured meats, however, present a more selective growth environment, which together with processing techniques, determine not only the saprophytic vegetative flora, but also the survival and/or growth of pathogens. The microbial stability and safety of cured meats can not be ascribed to a single factor (Baird-Parker and Baille, 1973). Bacterial inhibition depends mainly on the interaction of pH, nitrite, NaCl concentration, oxygen partial pressure, competition and temperature (Lechowich, 1971). These factors are interrelated, but they will be considered as single entities to stress their effects.

A. NaCl and Water Availability (Aw).

Microorganisms require available water for growth (Christian and Waltho, 1962; Dempster, 1976; Lechowich, 1971), and growth occurs readily if the Aw of the substrate is favourable for the multiplication of the organisms involved (Leistner and Rodel, 1974). Aw has been defined as the ratio of water vapour pressure over a food to that over pure water at the same temperature (Mossel, 1974; Dempster,

1976; Christian and Waltho, 1962). Leistner and Rodel (1974) indicated that since the Aw of meat and meat products influences reproduction, metabolic activity and survival of the cells, by adjusting the Aw, the stability and safety of the product can be improved.

Most organisms predominating in meat and meat products grow at high Aw, with only a few requiring reduced Aw for growth (Leistner and Rodel, 1974). Food-borne bacterial pathogens generally grow at Aw levels of 0.83 to 0.999 (Troller, 1973). The optimum Aw for most organisms is in the range of 0.995 to 0.998 (Lechowich, 1971). However, Aw of 0.95 and greater support growth of gram negative bacteria, as well as bacilli and clostridia (Leistner and Rodel, 1974). Lactobacilli, pediococci and micrococci tolerated a lower Aw (Leistner and Rodel, 1974), while S. aureus is capable of growth at 0.86 under aerobic conditions (Christian and Waltho, 1962). Scott (1957) reported that Aw levels of 0.80 - 0.90 can be tolerated by the more resistant organisms of cured meats. Since the Aw of cured meats is about 0.90 - 0.95 (Ingram, 1962), adequate water is present for growth and the microbiological safety of these foods becomes important.

It is expected that if the Aw of meats is reduced by drying, then microbial growth will be impaired (Dempster, 1976). Salt is an essential ingredient in meat curing (Kramlich et al., 1973; Jensen, 1949), and it increases the osmotic pressure; dehydrates the food product; reduces

oxygen solubility; sensitizes the cell wall to carbon dioxide; and affects the activity of proteolytic enzymes (Dempster, 1976; Frazier, 1967; Kramlich et al., 1973; Lechowich, 1971). Salt, therefore, causes an increase in lag phase, a decrease in growth rate and reduced synthesis of cell substance (Dempster, 1976; Frazier, 1967; Lechowich, 1971). In the manufacture of cured meat products, salt is used as a brine solution (Crittenden, 1974; Kramlich et al., 1973). The primary purpose of this cover pickle or brine solution is to lower the water activity (Frazier, 1967).

Cooked cured meat products may contain 1.8 - 3% salt, i.e. Aw ≥0.98 (Nickerson and Sinskey, 1972). Lactic acid bacteria grow well and should multiply at the salt concentrations in cured meats (Allen and Foster, 1960). In fact, the lactic acid bacteria isolated from sliced, processed meats were reported to tolerate up to 6.5% NaCl (Allen and Foster, 1960).

Matches and Liston (1972) reported that salt concentrations that inhibited Salmonella at lower temperatures, would not do so at higher temperatures. Even though Salmonella and E. coli do not grow in salt concentrations >6%, indications are that Salmonella survive better than E. coli over a range of salt concentrations (6 to 20% NaCl) at 5 C (Dempster, 1976). However, Salmonella were reported to proliferate in the presence of 8% salt provided the temperature and pH are near optimum (Alford and Palumbo, 1969). Aw of 0.99 supports optimum growth of

salmonellae and any reduction in Aw results in increases in lag phase and total cell yield regardless of whether growth is aerobic or anaerobic (Christian and Scott, 1953). The minimum Aw at which Salmonella grows is around 0.94 (Davidson and Webb, 1973) to 0.95 (Leistner and Rodel, 1974), and no increase in numbers occurs in foods with a Aw <0.92 (Christian and Scott, 1953).

Baird-Parker et al. (1970) reported that the heat resistance of Salmonella in foods with Aw of 0.99 is independent of the heating menstruum, and that the addition of NaCl to a final concentration of 6.1% reduces the heat resistance of heat-resistant strains. The heat-sensitive strains, on the other hand, increase in heat resistance. Smith et al. (1975) cited a report in which the rate of decline of salmonellae inoculated onto dry sausages was shown to be a function of the pH, salt and water content. In cured hams with high salt content (4%), salmonellae are inhibited, but survive curing (Akman and Park, 1974). In ham with a low salt content (2.0%), certain salmonellae increased 10- to 100-fold within 24 h (Akman and Park, 1974). Davidson and Webb (1973) pointed out that the level of curing ingredients, pH and Aw values found in cooked, cured meats are within the limits for Salmonella growth.

Riemann et al. (1972) reported that the salt concentration of many meat products is insufficient to inhibit growth of S. aureus. However, Dempster (1976) cited a report which showed that there are marked variations in

the ability of different strains to produce enterotoxin in media containing salt, with one strain inhibited by 2% NaCl, and another producing toxin in 10% NaCl. S. aureus are reported to be one of the most salt tolerant food-borne pathogens, and they are selected by high salt conditions (Troller, 1973). An Aw of 0.86 is required under aerobic conditions to inhibit the growth of S. aureus (Christian and Waltho, 1962), but the production of enterotoxins B and C ceases at Aw 0.94 (Genigeorgis et al., 1971). Genigeorgis and Sadler (1966) reported that enterotoxin B could be detected in Brain Heart Infusion broth at pH 6.9, containing 10% NaCl, or at pH 5.1 in 4% NaCl. The conclusion drawn is that enterotoxin production is affected by pH and salt concentration.

C. perfringens grows in meat and meat products having a minimum Aw of 0.95 (Leistner and Rodel, 1974). Sporulation, however, occurs only at Aw of 0.99, while spore germination is inhibited at Aw 0.95 (Troller, 1973). Mead (1969) reported that several strains of C. perfringens are capable of growth in a medium containing 6% NaCl.

Mossel et al. (1967) investigating NaCl as a selective agent for B. cereus, concluded that 5% NaCl is needed for good growth. Sodium chloride concentrations up to 8% does not prevent germination of B. cereus spores, but concentrations of 10 to 15% NaCl progressively reduced, and finally prevents germination (Gould, 1964). Spore outgrowth only requires 4 - 5% NaCl to be inhibited (Gould, 1964).

Dempster (1973) stated that temperature has a controlling influence on the inhibitory action of salt, and that this action is less effective at low temperatures. Corry (1974) stated that the heat resistance of microorganisms observed at reduced Aw values is greater in strains that can bind the most water.

B. pH.

In cured meat products, acid production depends on the nature of the contaminating microflora (Dack and Lippitz, 1962; Gilliland and Speck, 1972; Hurst, 1973; Ingram, 1962; Riemann et al., 1972), as well as the amount of fermentable carbohydrate present (Kempton and Bobier, 1970; Riemann et al., 1972). Lactic acid is generally produced in vacuum packaged luncheon meats (Kempton and Bobier, 1970), by lactobacilli (Allen and Foster, 1960; Alm et al., 1961; Smith and Palumbo, 1973), streptococci (Gilliland and Speck, 1972; Daly et al., 1972), atypical streptobacteria (Mol et al., 1971), pediococci (Riemann et al., 1972), and other related lactic organisms. The accumulation of lactic acid generally results in a decrease in pH. The final pH attained in comminuted meat products is ≤5.0, while the pH in ham is much higher (Kempton and Bobier, 1970).

The acidity of many foods is adequate to prevent growth of food poisoning bacteria (Lechowich, 1971), but the pH of semi-preserved meats does not prevent the growth of pathogens, especially at elevated storage temperatures

(Riemann et al., 1972). However, mild degrees of acidity are sufficient to enhance the effect of heat on bacteria and spores (Lechowich, 1971). The inhibitory action of low pH on bacteria is attributed to direct toxicity of the acid (Daly et al., 1972; Nickerson and Sinskey, 1972), or to the interaction with other inhibitors (Buchanan and Solberg, 1972; Dack and Lippitz, 1962; Ingram, 1962; Lechowich, 1971; Riemann et al., 1972). In the presence of salt, decreasing pH values have a marked inhibitory effect on Salmonella (Alford and Palumbo, 1969). Less salt is needed to prevent bacterial growth as the pH decreases (Ingram and Kitchell, 1967), and Dempster (1976) cited a report which showed that bacteria resistant to salt, e.g. micrococci, are unusually sensitive to acidity, while those sensitive to salt are more resistant to acidity.

Most bacteria grow best near pH 7.0, with maximum and minimum values around pH 8.0 and 5.0, respectively (Frazier, 1967; Lechowich, 1971), but Peterson et al. (1964) reported a wider pH range for bacterial growth i.e. between pH 4.5 and 9.0. Variations in pH do not appear to influence the microbiology of dry cured hams (Graham and Blumer, 1971).

Microorganisms vary considerably in their response to acid conditions under both laboratory and natural conditions. Peterson et al. (1964) cited a review which stated that Lactobacillus bifidus grows best between pH 3.8 and 7.2, while Streptococcus pyogenes grows between pH 6.5 and 9.2. In reviewing pathogenic organisms in cured meats,

Dempster (1976) stated that E. coli rarely grow in 1% peptone at pH 4.8. In sterilized meat slurries adjusted with phosphoric acid, no growth of E. coli was observed below pH 4.5 (Dack and Lippitz, 1962).

Generally, pH 4.6 is the lower limit for growth of Clostridium botulinum (Lechowich, 1971). C. botulinum type E is more sensitive to pH than types A and B, and all types are less pH tolerant in laboratory media, with growth inhibited at pH 4.8 to 5.0 (Riemann et al., 1972). Since C. botulinum germinates and grows in a strongly acidic environment, reduction of pH below 5.0 ensures extra safety (Cragg and Andrews, 1973).

Spores of Clostridium perfringens germinates in the pH range 5.5 - 7.0 with maximum germination occurring at pH 6.0 (Ahmed and Walker, 1971).

In Lebanon bologna, low pH contributed to the destruction of Salmonella typhimurium, but these organisms are not killed completely even after 4 days fermentation (pH 4.3 - 4.4), and are detected in low numbers during mellowing of the product (Smith et al., 1975). If a starter culture is used, destruction of salmonellae is more efficient but not uniform, and no viable S. typhimurium cells are detected by the third day of natural fermentation (Smith et al., 1975). Salmonellae are able to initiate growth in Trypticase Soy broth adjusted to pH 5.0 with lactic acid (Goepfert and Chung, 1970), but Salmonella are not capable of growth in meat slurry acidified with phosphoric acid to pH 4.0 - 4.3

(Dack and Lippitz, 1962). Salmonellae are also able to multiply in Thuringer sausage emulsion which, after fermentation, has a final pH of 5.2 - 5.4 (Goepfert and Chung, 1970). S. typhimurium is the serotype most often isolated from cases of human salmonellosis (Goepfert and Chung, 1970), and this serotype appears to be more acid sensitive than other types (Smith et al., 1975).

Staphylococci grow over a narrower pH range than other bacteria (Peterson et al., 1964). Although low pH inhibit S. aureus (Peterson et al., 1964), this organism's response to acidity varies with the strain and is influenced by the type of acid (Genigeorgis et al., 1969). It was found that with decreasing pH in cured meats, a greater number of staphylococcal cells must be present for growth to be initiated, either aerobically or anaerobically (Goepfert and Chung, 1970; Lechowich, 1971). Aerobic growth is diminished at pH 5.3, while anaerobic growth is completely inhibited (Lechowich, 1971) indicating that less acid is required under anaerobic conditions (Goepfert and Chung, 1970). Food poisoning S. aureus grow vigorously in ground pork containing permitted levels of curing ingredients (5% NaCl; ≤200 ppm nitrite) and anaerobic growth is prevented even in the absence of nitrite if the pH of the meat is lowered to 4.8 - 5.0 (Lechowich et al., 1956). No growth of S. aureus occurs below pH 4.5 in meat pie slurries adjusted with phosphoric acid (Dack and Lippitz, 1962).

Although staphylococcal enterotoxin production is

possible in cured meats at relatively low pH values, production of enterotoxins B and C is limited at these values under anaerobic conditions (Riemann et al., 1972). In fact, enterotoxins B and C are only encountered at high pH values provided the brine concentration is low (Genigeorgis et al., 1971). Although a drop in pH inhibits growth of S. aureus, toxin can be elaborated before an inhibitory pH (<5.0) is attained (Dempster, 1976). Dempster (1976) stated that acid conditions do not destroy preformed toxin, therefore, lowering the pH only affords limited protection.

C. Partial Pressure of Oxygen and Carbon Dioxide.

The oxygen tension of a food can exert a significant influence on the type of organisms that grow (Ingram, 1962; Richardson, 1973), and the type of waste product that a given microbial population produces (Richardson, 1973). The partial pressure of gases in the internal atmosphere of the vacuum package depends on the composition of the atmosphere as well as its pressure, both changing with time (Ingram, 1962).

Thatcher et al. (1962) stated that vacuum packaging was being used with increasing frequency for specific processed foods. An anaerobic environment developed because of the utilization of oxygen by bacteria and meat enzymes (Ingram, 1962; Shank and Lundquist, 1963), and the accumulation of carbon dioxide (Anon., 1973).

Packaging can influence the safety of semi-preserved

meats (Riemann et al., 1972), because the small amount of oxygen remaining after commercial vacuum packaging is sufficient for the growth of many aerobic organisms. It was suggested that inhibition in impermeable packages may not be due to lack of oxygen, but rather to increased carbon dioxide concentration (Ingram, 1962). However, it has been stated that microorganisms vary in their reaction to increased carbon dioxide concentrations (van Cooten, 1973), and carbon dioxide was found to inhibit aerobic growth while not affecting lactic acid producing bacteria (Baran et al., 1970; Gardner et al., 1967).

Although vacuum packaging generally inhibits growth of aerobic bacteria, yeasts and moulds (Shank and Lundquist, 1963), the development of certain resistant types can occur (Richardson, 1973). Gardner et al (1967) indicated that the depletion of oxygen and the increase in carbon dioxide creates conditions that select for growth of M. thermosphactum. The exclusion of oxygen also retards the development of a spoilage microflora (Ingram, 1962). Vacuum packaging does nct inhibit or retard the development of lactic acid bacteria (Alm et al., 1961; Shank and Lundquist, 1963), but other microorganisms do not grow well (Miller, 1960).

Christiansen and Foster (1965) reported that vacuum packaging has little or no effect on the ability of C. botulinum to grow or produce toxin in cured meats. C. botulinum is anaerobic, carbon dioxide tolerant, and is

not inhibited under conditions created by vacuum packaging (Riemann et al., 1972). Hughes (1970) stated that C. perfringens introduced into vacuum packaged meats does not develop in these packages during the shelf life of the product at room temperature. The presence of curing salts, in particular nitrite, was reported to inhibit this organism (Hughes, 1970).

Vacuum packaging inhibits the growth of S. aureus, thereby reducing the likelihood of S. aureus food poisoning, especially in sliced ham (Christiansen and Foster, 1965). However, Hughes (1970) stated that S. aureus can grow in vacuum packaged meats because of their ability to withstand low oxygen tensions.

Salmonella apparently grow in vacuum packaged meats if the organism is introduced into the pack at the time of production, and if abusive storage temperatures are allowed (Davidson and Webb, 1973).

Alm et al. (1961) indicated that the shelf life of vacuum packaged products is greater when the initial bacterial counts are low, and that failure of anaerobes and other organisms to grow is not due to partial pressure of oxygen alone. For instance, nitrite is more effective in the absence of oxygen (Castellani and Niven, 1955; Ingram 1973), and Riemann et al. (1972) reported that S. aureus is inhibited by a combination of anaerobiosis, NaCl, refrigerated storage and pH.

D. Nitrite.

The nitrite used in cured meat products is responsible for the characteristic pink colour, flavour and the development of a superior product (Ingram, 1973; Kemp et al., 1974; Pivnick et al., 1970). In addition, nitrite aids in stabilizing these products against toxigenic and/or other food poisoning organisms, as well as putrefactive bacteria (Duncan and Foster, 1968a; Hill et al., 1973; Ingram, 1973; Pivnick et al., 1970). The amount of nitrite required is small (Nordin, 1969); 15 to 20 ppm is adequate for acceptable colour and flavour development (Hill et al., 1973).

Nitrite concentrations up to 600 ppm at pH 6.0 allows emergence and elongation of vegetative cells, but blocks cell division (Duncan and Foster, 1968b). Pivnick et al. (1970) reported that nitrite at commercially used levels (200 ppm) neither enhances destruction of spores of C. botulinum during heating, nor germination during incubation. However, at concentrations of nitrite between 400 - 1600 ppm, there is increased spore destruction by heat, and increased germination of more spores during post-heating incubation (Pivnick et al., 1970). Roberts and Ingram (1966) reported that spores given increased heat treatments are rendered more sensitive to subsequent inhibition by curing salt concentrations of the order found in cured meats. Nitrite concentrations of 50 ppm are reported to have an inhibitory effect on the outgrowth of

heated spores at pH 6.5 (Roberts and Ingram, 1966). However, Pivnick et al. (1967) reported that heat injury of spores apparently is not necessary for sodium nitrite to inhibit toxigenesis.

Cured meats in the marketplace are reported to have little or no residual nitrite (Pivnick et al., 1970). The reasons for this include: nitrite depletion (20 - 25%) during the mixing of raw meat ingredients (cited by Hill et al., 1973), and the progressive destruction of nitrite by the reducing systems especially in fresh pork (Rose and Peterson, 1953). Sauter et al. (1977) reported that 60% of the initial level of nitrite remains in meat held for 24 h, and only 10% remains after 4 weeks. Rapid reduction of nitrite also occurs during pasteurization (Nordin, 1969), with losses between 30 - 90% being reported by Grever (1973). Nitrite is unstable during storage with significant depletion occurring in comminuted ham stored for one week (Pivnick et al., 1967), and in frankfurters, sausages, salami and spiced luncheon meat stored up to 10 days (Hill et al., 1973). Losses of one-third to one-half of nitrite occurs in comminuted ham held for a few hours at 4 C and then cooked (Pivnick et al., 1967).

Storage temperature is also important, and losses of nitrite are 2 to 35 times greater at 30 C than at 20 C (Pivnick et al., 1967). The unexpectedly rapid depletion of nitrite in pork products at room temperature is probably due to bacterial utilization (Nordin, 1969). Hill et al. (1973)

cited a report which concluded that the rate constant for nitrite depletion is directly proportional to the meat:water ratio. The greater the water content the faster the depletion of nitrite.

Many mechanisms have been suggested to explain the bacteriostatic or inhibitory effect of nitrite. Nitrite is considered to be a general metabolic inhibitor that can adversely affect sulphur metabolism (Riha and Solberg, 1975), extend the lag phase (Buchanan and Solberg, 1972), decrease growth (Buchanan and Solberg, 1972; Riha and Solberg, 1975), damage cells (Buchanan and Solberg, 1972), and affect general cellular metabolism (Riha and Solberg, 1975). In addition, Shank et al. (1962) reported that nitric oxide under aerobic conditions causes complete inactivation of bacteria after 15 min.

Inhibition by nitrite increases under acid conditions (Duncan and Foster, 1968b; Ingram, 1973; Shank et al., 1962; Tarr, 1941; Lechowich, 1971), with nitrous acid cited as the active component (Ingram, 1973; Shank et al., 1962; Lechowich, 1971). It was also reported that smaller quantities of nitrite are needed to inhibit growth as the pH decreased (Baird-Parker and Baille, 1973; Ingram, 1973; Lechowich, 1971).

The antimicrobial effect of nitrite in cured meats cannot be considered in isolation. Nitrite is more inhibitory under anaerobic conditions (Castellani and Niven, 1955), and the antimicrobial effect increases with

decreasing pH to a maximum near pH 5.0 (Shank et al., 1962). Inhibition also increases with increasing concentrations of nitrite present before the heating process (Chang and Akhtar, 1974). The inhibitory effect of nitrite alone, and nitrite and salt together is also dependent on pH (Baird-Parker and Baille, 1973; Shank et al., 1962).

Since depletion of nitrite occurs when it is added to meat systems (Nordin, 1969), dormant spores can grow after its depletion. This does not necessarily mean that all antimicrobial activity is terminated, especially since nitrite can be converted into other substances with inhibitory properties (Ingram, 1973). For instance, meat heated with nitrite can form a Perigo-type inhibitor (Chang and Akhtar, 1974; Pivnick and Chang, 1973). Considering the labile nature of nitrite it may be concluded that commercially cured, cooked processed meats contain anywhere from 1 - 200 ppm nitrite (Lechowich, 1971).

There is marked variation in the susceptibility of different bacteria to nitrite. Lactobacilli, pediococci and S. lactis were shown to be relatively tolerant to nitrite, especially under aerobic conditions (Castellani and Niven, 1955). Grever (1973) indicated that clostridia can be inhibited by 100 ppm nitrite provided the brine is 3.5% and the heat treatment has a value Fo=0.5.[1] Some C. botulinum strains resist as much as 150 - 200 ppm nitrite at 25 C

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[1] Fo is a term normally used in process calculation work. Fo=0.5 means a heat process in effect equal to a heating of 0.5 min at 121 C

(Baird-Parker and Baille, 1973) and C. botulinum types A and B have been reported to grow in some cured, cooked luncheon meats (Christiansen and Foster, 1965; Steinke and Foster,1951). In contrast, nitrite inhibits C. perfringens (Perigo and Roberts, 1968), with 50 ppm causing reduced survival of spores inoculated in meats (Sauter et al., 1977).

Commercial levels of nitrite (142 ppm) are inhibitory to spores of bacilli in meat products (Mol and Timmers, 1970). B. cereus spores are reported to germinate at reduced rates in 300 ppm nitrite and not at all in 750 - 2500 ppm nitrite at pH 6.0 (Gould, 1964). After heating at 80 C for 30 min, B. subtilis spores are not inhibited by 1600 ppm nitrite at pH 7.6 or 6.6, whereas only 200 ppm is needed for inhibition at pH 5.6 (Roberts and Ingram, 1966). Grever (1973) also found that to prevent the growth of bacilli more nitrite is required than that needed to prevent growth of clostridia. S. typhimurium is able to initiate growth at pH 5.0 and 5.5 in the presence of 100 and 200 ppm nitrite, and salmonellae multiplied in sausage emulsions not containing starter culture during fermentation (Goepfert and Chung, 1970). In contrast, 100 ppm nitrite reduces the growth of Salmonella enteritidis by about 30% at pH 5 in 5% NaCl (Akman and Park, 1974). Tarr (1941) reported that at pH 5.7 to 6.5, 200 ppm nitrite is enough to inhibit the genus Escherichia.

Nitrite is inhibitory to S. aureus at levels permitted

in food products such as cooked, cured meats at the pH of 6.3, which is the expected value for these products (Buchanan and Solberg, 1972). However, 200 ppm nitrite has no effect on growth or enterotoxin production in broth (McLean et al., 1968), and 2,000 ppm nitrite does not affect growth during aerobic incubation at pH 7.3 (Buchanan and Solberg, 1972). It was found that nitrite is a more effective inhibitor of S. aureus under anaerobic conditions than under aerobic conditions (Castellani and Niven, 1955).

E. Competition.

Uncontrolled bacterial growth in foods is considered disturbing because conditions which support growth of spoilage bacteria, can allow food poisoning organisms to develop (Hurst, 1973). Hurst (1973) stated that microbial antagonisms in foods have been documented for raw and semi-preserved meats as well as in thawing foods.

Several mechanisms for microbial competition have been suggested:

(a) production and liberation of antibiotic substances;

(b) exhaustion of essential nutrients;

(c) hydrogen peroxide and organic acid production;

(d) physicochemical changes i.e. decreases in oxidation-reduction potential;

(e) enzyme activity and spore antagonisms (Troller and Frazier, 1963; Gilliland and Speck, 1972; Hurst 1973; Anon., 1973).

The natural or saprophytic flora of cured meats was reported to be antagonistic to bacteria of public health concern, in particular S. aureus (Genigeorgis et al., 1969; Peterson et al., 1964; Eddy and Ingram, 1962;). Dack and Lippitz (1962) reported that the natural flora of frozen pot pies exerts an inhibitory effect on S. typhimurium and E. coli.

With respect to the saprophytic flora, enterococci are seen to be the most effective competitors. Hurst (1973) stated that enterococci are inhibitory to gram negative bacteria, and Kafel and Ayres (1969) reported that they are antagonistic towards selected species of bacilli, clostridia, lactobacilli, and other associated microbes inoculated onto hams. Of the enterococci examined, S. faecalis showed the strongest antagonism (Kafel and Ayres, 1969). It has been suggested that the inhibitory action of the enterococci is not one of direct antagonism, but is caused by the production of an antibiotic such as nisin (Kafel and Ayres, 1969). Hurst (1973) stated that S. lactis produces the antibiotic nisin, which has wide application.

Salmonellae and staphylococci have been reported in foods cultured with lactic streptococci, but the degree of antagonism could not be predicted by the rate of acid production (Gilliland and Speck, 1972). The associated growth of S. lactis and E. coli type I at 30 and 37 C resulted in the rapid disappearence of E. coli from the

mixtures, even though it was originally the dominant organism in some samples (Hall, 1957). S. diacetilactis can repress S. aureus by depleting the essential nutrient, nicotinamide (Iandolo et al., 1965), and inhibits a variety of food spoilage organisms and pathogens (Pseudomonas, E. coli, Salmonella, C. perfringens, and S. aureus) by a combination of low pH, acid production and possible antibiotic elaboration (Daly et al., 1972). In addition, Gilliland and Speck (1972) stated that streptococci produces hydrogen peroxide in sufficient amounts to affect the growth of food-borne pathogens.

Hurst (1973) stated that lactobacilli are capable of causing inhibition by virtue of acid, hydrogen peroxide and antibiotic production. Lactobacillus acidophilus exerts antagonistic actions on the growth of S. aureus, S. typhimurium, E. coli, and C. perfringens when grown with each in associative culture (Gilliland and Speck, 1977). The amount of antagonism did not appear to be directly related to the amount of acid produced, and was due in part to hydrogen peroxide production. The inhibitory effect was also produced under anaerobic conditions in a pre-reduced medium (Gilliland and Speck, 1977).

Gilliland and Speck (1972) cited data which showed that when leuconostocs are included in starter cultures, they inhibit Salmonella gallinarium and other gram negative bacteria. Troller and Frazier (1963) reported that Serratia marcescens and Pseudomonas spp. inhibit S. aureus by

competing for nutrients, in particular, amino acids, while B. cereus and E. coli are inhibited by an antibiotic substance.

Enteropathogens are also inhibitory to each other. For example, Mickelson and Flippin (1960) reported the use of an E. coli strain to eliminate salmonellae from egg white. E. coli was also reported to produce an antibiotic which is antagonistic to S. aureus and micrococci (Troller and Frazier, 1963). Hurst (1973) cited reports which indicated that S. aureus is capable of producing antibiotics which are effective against other bacteria e.g. other staphylococci and micrpcocci.

The majority of S. aureus food poisonings are reported to occur in foods in which the microbial flora has been reduced, and there is an absence of microbial competition. Under these conditions, it has been stated that S. aureus grew well and elaborated toxins, even under anaerobic conditions (Anon., 1973).

F. Temperature.

Temperatures employed in processing and storage procedures would affect the microorganisms in cured meat products. Pasteurization of ham and other semi-preserved meats not only causes binding of the chunks, but also reduces total bacterial loads (Gardner, 1968). For instance, smoking to an internal temperature of 60 C causes a major reduction in the number of microorganisms in frankfurters

(Heiszler et al., 1972).

Lactic acid bacteria can survive processing at 70 C for 10 min (Gardner, 1968), and many strains of lactobacilli isolated from meats were extremely heat resistant e.g. strains of L. viridescens can withstand 120 min at 65 C (Sharpe, 1962). Hence these organisms can survive heat processing. Furthermore, good growth of lactic acid bacteria has been reported at <10 C (Bartl, 1973), while slower growth has been reported at -1 to 3 C (Jaye et al., 1962). Typical psychrotrophic food spoilage bacteria develop faster at marginal refrigeration temperatures (7 - 10 C) than at 1 - 3 C (Allen and Foster, 1960), or at -1 C (Jaye et al., 1962).

The ability of the enterococci to withstand but not grow at elevated temperatures was reported by Shannon et al. (1970). In fact, some enterococci survive exposure to 62.8 C for 30 min in skimmilk (Shannon et al., 1970). Shannon et al. (1970) cited a report which indicated that since S. faecium is capable of surviving the minimum processing temperatures for canned hams, internal temperatures of 70 C are needed to reduce the potential spoilage hazard.

Food poisoning organisms have been stated to be mostly mesophiles (Richardson, 1973) and have optimum growth temperatures of 35 - 40 C, with a minimum of 10 - 15 C (Stanier et al., 1970). However, certain mesophiles, including pathogens, can grow slowly at 5 - 10 C (Angelotti et al., 1961; Tatini, 1973). A thermal process equivalent to

60 C for 100 min eliminates salmonellae including heat resistant strains (Davidson and Webb, 1973). In addition, it was found that an internal temperature of 52 C for 1 h decreases the population of S. typhimurium inoculated onto sausage products by more than 99.99% (Goepfert and Chung, 1970), and the introduction of an optional cooking step indicates that heating of bologna to 51.7 C or above leads to destruction of S. typhimurium. In addition, Angelotti et al. (1961a) reported that no salmonellae grow in foods held below 5 C. However, these organisms apparently survive extended storage. For example, samples of ham inoculated with S. typhimurium and stored at 7 C contained viable organisms even at 11 weeks (Davidson and Webb, 1973), and viable salmonellae are recovered from sliced, vacuum packaged sausage after 42 days at 5 C (Goepfert and Chung, 1970). Goepfert and Chung (1970) concluded that refrigation does not free these products from Salmonella contamination.

Any method of cooking where the temperature is not greater than 100 C allows the survival of spores of C. perfringens (Hobbs, 1969). Spores of C. perfringens were shown to survive cooking (Sutton et al., 1972) and heat treatment at 75 C for 20 min was generally needed for spore activation (Ahmed and Walker, 1971). Germination can occur in the temperature range of 7 - 46 C with an optimum at 30 C (Ahmed and Walker, 1971). Although vegetative cells of C. perfringens do not survive cooking (Sutton et al., 1972), Gough and Alford (1965) emphasized the persistence of these

organisms in hams subsequent to both curing and smoking. C. perfringens was reported to have an optimum growth temperature of 43 - 47 C, with growth restricted below 15 - 20 C, and no growth reported below 6.5 C, even after 7 days (Hobbs, 1969).

Heat processing destroys vegetative cells of C. botulinum in processed meat, but types A and B can not grow at refrigeration temperatures (Pivnick et al., 1967). Riemann et al. (1972) cited a report which indicated that proteolytic strains of C. botulinum do not multiply below 10 - 12 C, while the non-proteolytic strains grow and produce toxin slowly at 3 C.

Staphylococci are heat sensitive (Anon., 1973), and these organisms are killed when hams are heated to an internal temperature of 58 C (Lechowich et al., 1956). It was also stated that staphylococci could grow between 6.7 and 45.5 C (Anon., 1973). In fact, enteropathogenic S. aureus grow well at 10 C (Evans and Niven, 1950). It was stated that aerobic enterotoxin production has been reported between 15.5 - 45.5 C, while anaerobic enterotoxin B production is possible in cured meats incubated at 10 C. (Anon., 1973). Better toxin production is possible in laboratory cured hams, inoculated with S. aureus and incubated at 30 C, than at 22 or 10 C. (Genigeorgis et al., 1969). At 10 C, toxin was only detected after at least 2 weeks incubation, and only after 8 weeks when the pH was greater than 5.6 (Genigeorgis et al., 1969).

In a review, Hobbs (1969) stated that B. cereus grows between 10 and 45 C, with 10 C, 30 - 35 C and 49 C being the minimum, optimum and maximum growth temperatures, respectively. It was also stated that the spores are fairly resistant to heat (Hobbs, 1969).

High storage temperature accelerates growth of pathogens, and with reduced competition, growth is further encouraged (Paradis and Stiles, 1978b). However, the presence of inhibitory substances possibly restricts the growth of pathogens.

4. Epidemiological Information

A. S. aureus.

The high frequency of implication of cured meats in S. aureus food poisoning has been attributed to the tolerance of S. aureus to NaCl concentrations inhibitory to other organisms (Hobbs, 1965). However, Hodge (1960) suggested that even though S. aureus are ubiquitous, and many strains are enterotoxigenic, the mere presence of these organisms in foods is not sound evidence for the diagnosis of this disease. Hodge (1960) also stated that staphylococcal food poisoning is the most prevalent food-borne disease in the United States. Minor and Marth (1971) reported that S. aureus food intoxications rank first in incidence among reported outbreaks of food-borne diseases in the United States during 1970, and third in incidence of all reported cases. S. aureus was responsible for more

food-borne incidents (33), and more cases (606) than any other agent in Canada in 1973 (Todd, 1976).

A wide range of food vehicles have been implicated in staphylococcal food poisoning, including: macaroni, potato and other salads (Pace, 1975), sausage (Minor and Marth, 1971), ham slices (Eisenberg et al., 1975), and sandwiches (Christiansen and King, 1971; Morris et al., 1972). Inadequately refrigerated ham, deboned chicken and trifles have also been implicated in staphylococcal food poisonings (Anon., 1976). Minor and Marth (1972) reported that the vehicles of several staphylococcal food poisoning outbreaks were meat and bakery products. In a study of 926 outbreaks of diarrhoea, dysentery, food poisoning and gastroenteritis, Fieg (1950) indicated that of meat products, ham was the greatest single offender. Also, Dack (1962) stated in a review that in the United States from 1956 to 1961, 57 of the 137 food poisoning cases due to S. aureus were associated with meat, and 45 of these cases were from hams. In England and Wales, 47 of 85 staphylococcal food poisonings from 1959 to 1963, were due to manufactured meats such as ham and bacon (Hobbs, 1965). In Canada, of the 118 food-borne incidents due to meats in 1973, only 19 incidents were attributed to ham and pork (Todd, 1976).

B. Salmonella.

Salmonellosis was cited as a major food-borne hazard to human health (Childers et al., 1973). Of 69 incidents of

microbiological food poisoning in Canada in 1973, 14 incidents (334 cases) were due to Salmonella (Todd, 1976). Bryan (1974) pointed out that summaries from the U.S. Centre for Disease Control showed that 1,703 outbreaks of food-borne disease (97,590 cases) were reported during 1968 to 1972, and salmonellosis was one of the diseases most frequently reported. Hobbs (1973) reported an average of 6,000 outbreaks of food poisoning per year in England and Wales between 1968 and 1970, and that salmonellae were responsible for the majority of outbreaks. Before that, in 1966, S. typhimurium was responsible for 1,407 outbreaks (2,346 cases) of food poisoning (Hobbs, 1969). Angelotti (1969) also reported that Salmonella was responsible for 92 to 95% of the food poisonings in the U.K. during 1961 to 1963.

After personal communication with B.C. Hobbs, Central Public Health Laboratory, London, Davidson and Webb (1973) reported that in Salmonella food poisoning, the vehicle was identified in only 16% of the general outbreaks in the U.K., and stated that the available data indicated that vacuum packaged ready-to-eat, cured meats were seldom associated with salmonellosis. Although cooked, cured pork was rarely associated with salmonellosis (Bailey et al., 1972), this product could be a source of Salmonella food poisoning because of the trend toward lower salt concentrations during production (Akman and Park, 1974). However, Smith et al. (1975) reported that outbreaks of salmonellosis were rarely

attributed to sausages. It appeared that the number of viable Salmonella cells in food that resulted from contamination was insufficient to cause salmonellosis (Idziak and Crossley, 1973), and that multiplication must follow contamination in order for salmonellosis to occur (Akman and Park, 1974).

C. C. perfringens.

Food poisoning outbreaks due to C. perfringens remained common (Thatcher, 1963). However, Riemann et al. (1972) stated that C. perfringens has seldom been reported in food poisoning from semi-preserved meats. Todd (1976) reported that 7 of 57 outbreaks of microbiological food poisoning in Canada in 1973 could be attributed to C. perfringens. In the United States, this organism accounted for 28% of the outbreaks in 1968. In England and Wales, in 1966, C. perfringens was responsible for 63 outbreaks which included 1,947 cases (Hobbs, 1969). Beef and poultry were the most common vehicles of C. perfringens food poisoning (Duncan, 1970).

D. B. cereus.

Food poisoning by B. cereus is similar to C. perfringens (Foster, 1973). Goepfert et al. (1972) in their review of B. cereus food poisoning cited one report which stated that B. cereus was ranked third as the causative agent of food poisoning in Hungary. Another report

stated that B. cereus accounted for 8.2% of the outbreaks between 1960 and 1968. In 1969, Angelotti (1969) noted that there were numerous cases of B. cereus food poisoning in the Netherlands. B. cereus food poisoning remains an unknown quantity in the United States compared to the large number of cases recorded in Europe (Goepfert et al., 1972).

The most common vehicles reported for B. cereus food poisoning were meats, including sausages and sauces which were exposed to temperature abuse (Goepfert et al., 1972). Hauge (1955) stated that B. cereus food poisoning outbreaks have occurred mainly in connection with dinners in large institutions like hospitals and boarding houses.

E. E. coli.

Hall et al. (1967) stated that the importance of enteropathogenic E. coli has rarely been considered in the United States except when all environmental factors were being studied in outbreaks of infantile diarrhoea. Taylor (1955) stated that reports of food poisoning due to E. coli are fairly numerous, but that the evidence implicating this organism is unsatisfactory. However, the evidence presented so far has been against a casual role for E. coli, and it is generally accepted that certain serotypes of E. coli can cause infantile diarrhoea.

III. Microbial Quality of Vacuum Packaged, Sliced Ham

1. Abstract

A total of 120 samples of vacuum packaged sliced ham were purchased and analyzed to represent new (<10 days from manufacture) and old (pull date) product. Microbial loads differed between manufacturers, and with age of product. Lactic acid bacteria appeared to predominate the population. Indicator and potentially pathogenic bacteria were absent, or present in low concentration, suggesting that product from six federally inspected plants was safe, despite high total microbial loads. pH was poorly correlated with total microbial load and pH drop between new and old samples differed markedly between manufacturers.

2. Introduction

Vacuum packaged, sliced ham is a product of modern food processing. It is an integral meat, produced by massaging or tumbling of the meat to form a proteinaceous exudate (Crittenden, 1974), then "forming" the ham by moulding, and cooking to an internal temperature of 68 C (Anon., 1974; Woolen, 1971). Ham produced by this modern method requires shorter processing time (Crittenden, 1974); curing efficiency is increased (Crittenden, 1974; Weiss, 1974; Woolen, 1971); yields and final product texture are improved (Anon., 1977; Crittenden, 1974; Weiss, 1974); and it provides a convenient product for consumers and the food service industry (Crittenden, 1974).

Cooking virtually eliminates the lactic acid bacteria (Kempton and Bcbier, 1970). However these products are not sterile (Richardson, 1973), and in addition, slicing and packaging results in recontamination of the product (Allen and Foster, 1960; Kempton and Bobier, 1970; Lechowich, 1971). The initial microflora of sliced, processed meats is heterogeneous (Alm et al., 1961), however gram positive organisms have been shown to be selected by heat treatment and curing agents in frankfurters (Heiszler et al., 1972). Vacuum packaging also has a selective effect (Reuter, 1973; Sharpe, 1962). Enterococci and Lactobacillus spp. were reported to predominate in vacuum packaged sliced ham (Surkiewicz et al., 1977). A succession of microorganisms has been reported in bacon (Ingram and Dainty, 1971), first micrococci, then enterococci and finally lactic acid bacteria. The predominance of lactic acid bacteria in cured meat products has been widely reported.

A low incidence of pathogenic bacteria has been reported for vacuum packaged, cooked meat products (Paradis and Stiles, 1978a; Surkiewicz et al., 1977). It has been reported that salmonellae do not grow in vacuum packaged cured meats (Angelotti et al., 1961). However, it was concluded that a Salmonella food poisoning potential could exist with certain processed products, if they became contaminated and were held at abusive storage temperatures for long periods (Davidson and Webb, 1973). Although Clostridium perfringens has been isolated (Lechowich, 1971;

Strong et al., 1963), it has not been implicated in food poisoning outbreaks in these meats (Ingram, 1973). These meats also have the potential to be vehicles of Bacillus cereus food poisoning (Goepfert et al., 1972), but Staphylococcus aureus has been indicated as the principal health hazard in vacuum packaged meats (Hughes, 1970).

Although Paradis and Stiles (1978a) and Surkiewicz et al (1977) have reported a low incidence of pathogens, it is apparent that vacuum packaged, processed meats have the potential to be hazardous. In addition, survey studies of these products available in the retail marketplace have indicated widely differing microbial loads. The object of this study then was to determine the microbial load and the apparent safety of vacuum packaged, sliced ham.

3. Materials and Methods

A. Sampling.

Vacuum packaged sliced ham representing the product of six federally inspected, Canadian establishments was purchased from 3 retail chain stores. A total of 10 paired samples was collected for each manufacturer, within 10 days of manufacture. One sample was analyzed immediately in the laboratory and designated "new" ham; the other was held at 4 C to manufacturer's pull date before analysis and designated "old" ham.

B. Sample Preparation.

An 11-g wedge was cut aseptically through all slices in the package, and homogenized with 99 ml of sterile, 0.1% peptone water in a Waring Blendor jar at high speed for 2 min. All bacterial analyses were done on this homogenate, except Salmonella, for which a separate 25-g wedge was homogenized in 150 ml of sterile nutrient broth (Difco) for non-selective enrichment.

C. Bacteriological Analyses.

Appropriate dilutions of sample homogenates were plated in duplicate onto the selective and non-selective media listed in Table 1. For Salmonella determinations, the non-selective enrichment was subcultured into Selenite Cystine broth (Difco), incubated at 35 C for 24 and 48 h, and streaked onto Brilliant Green agar (Difco). Most probable numbers (MPN) of coliforms and presumptive Escherichia coli were determined using Lauryl Tryptose broth and EC medium in a 3-tube modification (ICMSF, 1978) of the Canadian Health Protection Branch method (HPB, 1974b). The S. aureus count on Baird-Parker medium was determined using EDTA coagulase plasma (Difco) in the HPB (1974a) technique.

D. Confirmation Tests.

Group D streptococci were confirmed using Bile Esculin agar (Facklam and Moody, 1970). Lactic acid bacteria were identified by morphological difference and confirmed using gram stain and cell morphology. Suspected Salmonella

colonies on Brilliant Green agar were screened on MacConkey agar and by biochemical tests.

E. pH.

Sample pH was determined using a combination, single electrode (Fisher Scientific Co., Cat. No. 1363990) by direct contact between the slices.

F. Analyses.

Data were analysed using a computerized statistical package (Nie et al., 1975) to determine Pearson's correlation coefficients, analysis of variance (ANOVAR) and Duncan's multiple range test.

4. Results

A total of 60 paired (new and old) samples of vacuum packaged sliced ham, representing product of six manufacturers, was analyzed. The distribution of pH values is shown in Table 2. New product was generally pH 6.00 and above. The pH decreased with age, but only 46.6% of old samples had pH <6.00. The pH drop was greatest in product from manufacturers B and F.

The distribution of total and lactic acid bacteria counts is shown in Table 3. Total microbial load and lactic acid bacteria counts increased with age. New ham samples had counts ranging from 10^1 to 10^6 per g, with a few samples up to 10^7 and 10^8 per g. Old samples had counts ranging from

Table 1. Media and incubation procedures used to determine saprophytic, indicator and pathogenic counts on vacuum packaged, sliced ham samples obtained from retail stores.

Count	Code	Media and Incubation Conditions
Standard Plate count	SP 35	Plate Count Agar (Difco), 35 C, 48 h.
Total Aerobic count	SP 21	Plate Count Agar (Difco), 21 C, 72 h.
Psychrotroph count	PSY	Plate Count Agar (Difco), 4 C, 10 days.
Presumptive Group D Streptococci	KF	KF Streptococcus Agar (Difco), 35 C, 24 and 48 h.
Lactic Acid Bacteria	APT	APT Agar (Difco), 30 C, 48 h.
Lactobacilli	LBS	LBS Agar (Rogosa's) (Difco), 30 C, 48 h.
Coliform count	VRBA	Violet Red Bile Agar (Difco), 35 C, 24 h.
Presumptive *E. coli* count	LST	Lauryl Tryptose Broth (Difco), 35 C, 24 and 48 h; and gas positive tubes into EC medium.
	EC	EC medium (Difco), 45.5±0.05 C, 24 and 48 h.
Micrococci	PCA10	Plate Count Agar (Difco), +10% NaCl (Mol *et al.*, 1971) 30 C, 48 h.
M. thermosphactum	STAA	Streptomycin Sulphate-Thallous acetate-Actidione agar 21 C, 48 h (Gardner, 1966).
B. cereus	MYP	Phenol Red, Egg-yolk, Polymyxin agar (Mossel *et al.*, 1967), 30 C, 24 h.
S. aureus	BP	Baird-Parker medium (according to Holbrook *et al.*, 1969), 35 C, 48 h.

Table 2. pH profiles of 60 paired ham samples (new and old) included in the retail survey

	pH range					
	5.25 -5.49	5.50 -5.74	5.75 -5.99	6.00 -6.24	6.25 -6.49	6.50 -6.74
	Number (%) of samples					
All samples	1	9	19	36	44	11
New Samples		1 (1.7)		14 (23.3)	34 (56.7)	11 (18.3)
Old Samples	1 (1.7)	8 (13.3)	19 (31.7)	22 (36.7)	10 (16.7)	
Manufacturer A						
New				3	6	1
Old			1	4	5	
Manufacturer B						
New				2	6	2
Old	1	2	6	1		
Manufacturer C						
New				1	7	2
Old			1	8	1	
Manufacturer D						
New		1		2	7	
Old		2	4	3	1	
Manufacturer E						
New					4	6
Old		1	1	5	3	
Manufacturer F						
New				6	4	
Old		3	6	1		

10^6 to 10^{10} per g, with a few old samples having SP 35 counts from 10^3 to 10^5 per g. In contrast, LBS counts covered a wide range, with a tendency toward increased counts with age, but this was by no means the general rule.

Additional microbial count distributions are shown in Table 4. All of these counts, except EC, increased with age of product, but increases were not as dramatic as those shown for lactic acid bacteria. These results suggested the predominance of lactic acid bacteria in the total microbial flora.

Group D streptococci and coliforms were detected at or above the minimum detectable level (10 organisms per g) in 45.0% of new and 51.7% of old samples. However, presumptive *E. coli* were not detected at their minimum detectable level (3/10 g) in any samples. Neither micrococci (PCA10) nor *Microbacterium thermosphactum* (STAA) predominated the microflora, although STAA counts ranged up to 10^7 per g, and generally increased during storage. PCA10 counts only increased slightly with storage at 4 C to pull date.

B. cereus and *S. aureus* were not detected at their minimum detectable levels of 100 per g, and *Salmonella* were not detected in 25-g of product using the enrichment techniques described.

The correlation of pH and the different microbial counts is shown in Table 5. High correlation coefficients were observed between APT and total counts (SP 35, SP 21 and

Table 3. Profiles of total and lactic acid bacteria counts on 60 new and old vacuum packaged, sliced ham samples.

Bacteria Counts		Range of Bacterial Counts per gram								
		10^1- $<10^2$	10^2- $<10^3$	10^3- $<10^4$	10^4- $<10^5$	10^5- $<10^6$	10^6- $<10^7$	10^7- $<10^8$	10^8- $<10^9$	$\geq 10^9$
		% of Samples								
SP 35	New		11.7	20.0	26.7	25.0	15.0	1.7		
	Old			3.3	1.7	3.3	8.3	56.7	18.3	8.3
SP 21	New		10.0	6.7	25.0	25.0	23.3	8.3	1.7	
	Old						1.7	31.7	46.7	20.0
PSY	New		13.3	6.7	26.7	23.3	20.0	8.3	1.7	
	Old					1.7	6.7	30.0	38.3	23.4
APT	New	3.3	8.3	10.0	26.7	28.3	20.0	1.7	1.7	
	Old						6.7	33.3	48.3	11.7
LBS	New	31.7	18.3	23.3	5.0	18.3	0.0	3.3		
	Old	16.7	1.7	3.3	3.3	6.7	16.7	38.8	10.0	3.3

Table 4. Profiles of indicator and selected adventitious bacteria counts on 60 paired (new and old) vacuum packaged, sliced ham samples.

Bacteria Counts		Range of Bacterial Counts per gram						
		$<10^1$	$10^1-<10^2$	$10^2-<10^3$	$10^3-<10^4$	$10^4-<10^5$	$10^5-<10^6$	$10^6-<10^7$	$\geq 10^7$
		% of Samples							
KF	New	55.0	25.0	15.0	1.7	1.7	1.7		
	Old	48.3	13.3	21.7	15.0	1.7			
VRBA	New	55.0	28.3	8.3	6.7	1.7			
	Old	48.3	16.7	11.7	8.3	13.3	1.7		
STAA	New		25.0	11.7	11.7	18.3	18.3	13.3	1.7
	Old		15.0	10.0	13.3	21.7	20.0	11.7	8.3
PCA10	New		50.0	35.0	6.7	6.7	1.7		
	Old		48.3	31.7	15.0	3.3	1.7		
EC	New	100							
	Old	100							

PSY). However, these counts were not highly correlated with pH, and correlations between pH drop and increase in APT counts for each manufacturer were low and not significant. Other correlation coefficients were generally low ($r < 0.7$).

A complete factorial analysis of variance (ANOVAR, Nie et al., 1975) was done to determine the effect of manufacturer and age of product on the different microbial counts. Manufacturer had a significant effect ($p < 0.001$) on all counts, while age had a significant effect on all counts ($p < 0.001$), except PCA10 ($p = 0.984$), STAA ($p = 0.068$) and KF ($p = 0.065$). Manufacturer and age interaction effects were limited and significant effects were only observed for SP 21 ($p = 0.013$), APT ($p = 0.004$) and pH ($p < 0.001$). These interaction effects were attributed to unusually high SP 21 and APT counts on new product from manufacturer C, and relatively high pH on old product A and low pH on old product B.

Results for further analysis of manufacturer effect using Duncan's multiple range test are shown in Table 6. Bacterial counts on product from manufacturer A were lower and generally significantly different for all counts tested. In contrast, manufacturer D had the highest counts on most of the media, however these counts were generally not significantly different from counts on other manufacturers'

Table 5. Pearson's correlation coefficients (r) between pH and various saprophytic and indicator bacteria counts for all (new and old) ham samples.

Variable	pH	SP 35	SP 21	PSY	KF	APT	LBS	STAA
SP 35	-0.524 (0.001) *							
Sp 21	-0.493 (0.001)	0.845 (0.001)						
PSY	-0.543 (0.001)	0.895 (0.001)	0.891 (0.001)					
KF	-0.189 (0.039)	0.262 (0.004)	0.201 (0.028)	0.291 (0.001)				
APT	-0.537 (0.001)	0.903 (0.001)	0.908 (0.001)	0.955 (0.001)	0.259 (0.004)			
LBS	-0.475 (0.001)	0.681 (0.001)	0.616 (0.001)	0.671 (0.001)	0.264 (0.004)	0.689 (0.001)		
STAA	0.088 (0.340)	0.363 (0.001)	0.393 (0.001)	0.406 (0.001)	0.232 (0.011)	0.353 (0.001)	0.116 (0.207)	
PCA10	-0.024 (0.792)	0.273 (0.003)	0.284 (0.002)	0.267 (0.003)	0.382 (0.001)	0.264 (0.004)	0.289 (0.001)	0.322 (0.001)

* level of probability

products, except manufacturer A. Product from manufacturer A did not have the highest pH, but pH was significantly higher than product from manufacturers B, D and F. Mean pH drop for product from manufacturer A was 0.11, and for manufacturers C, D, E, F and B was 0.24, 0.35, 0.40, 0.45 and 0.59, respectively.

5. Discussion and Conclusions

Old samples (30 ± 2 days from manufacture) could not always be obtained in the marketplace, so paired new samples were purchased within 10 days of manufacturer, and one of each pair held to the manufacturers' designated pull date for analysis. As a result, data for new samples represent typical product in the marketplace, but data for old samples represent product held to pull date under ideal storage conditions.

The wide range of total bacterial counts in new product suggested that product handling in the retail marketplace was quite variable. In new ham samples (≤10 days from manufacture), 40 - 50% of total and lactic acid bacteria counts exceeded 10^5/g (Table 3), indicating that temperature abuse occurred in some samples. Under idealized conditions of storage (30 ± 2 days at 4 C), total count populations ranged up to 10^9 to 10^{10}/g. However, the majority of the samples had total counts of 10^7/g (SP 35) and 10^8/g (SP 21 and PSY) similar to other reports (Allen and Foster, 1960; Hill et al., 1976; Ingram, 1962; Kempton and Bobier, 1970;

Table 6. Rank order of manufacturer means for bacterial counts and pH of vacuum packaged, sliced ham.

Bacterial Count and pH	Duncan's Multiple Range Test (5% confidence)*					
SP 35	A	B	E	C	F	D
SP 21	A	B	C	E	F	D
PSY	A	B	C	E	F	D
KF	A	E	F	B	C	D
APT	A	B	C	E	F	D
LBS	A	C	B	E	D	F
VRBA	A	F	B	D	C	E
STAA	A	F	B	D	E	C
PCA10	A	F	B	E	C	D
pH	F	B	D	C	A	E

* A common line under symbols representing different manufacturers indicates no significant difference between the means.

Warnecke et al., 1966).

APT counts were highly correlated with total counts (SP 35, SP 21 and PSY). APT agar is non-selective, but lactic acid bacteria overgrow and predominate on this medium (Kempton and Bobier, 1970). It may be concluded, therefore, that predominating bacteria in these ham samples were lactic acid bacteria, in agreement with reports for other vacuum-packaged meat products, including ham (Allen and Foster, 1960; Mol et al., 1971; Shank and Lundquist, 1963; Warnecke et al., 1966). In contrast, lactobacilli did not often predominate the population, contrary to reports by Alm et al. (1961), Foster (1959), Lechowich (1971) and Reuter (1973). Therefore, it could be concluded that the majority of lactic acid bacteria were the unclassified streptobacteria, confirming the findings of Mol et al. (1971). The STAA count, representing M. thermosphactum, did not represent a major part of the microflora, as might be expected from reports on factors influencing growth of this organism (Gardner et al., 1967).

Despite the predominance of lactic acid bacteria in these ham samples, pH was variable with growth of the inherent microflora, as shown by the correlation coefficient of -0.537 between APT count and pH. The pH achieved appeared to be a function of manufacturer, and hence might be due to differences in buffering capacities of the products (Frazier, 1967; Ingram, 1962; Pearson, 1971), or other differences in products between manufacturers. Ham contains

less carbohydrate that most comminuted meat sausage products (Kempton and Bobier, 1970) and hence less acid production might be expected. pH is often cited as a protective factor in vacuum packaged luncheon meats, but results of this study and studies on bologna (Paradis and Stiles, 1978a) and chopped ham sausage (Stiles and Ng, 1979a) indicated that pH levels achieved in these products, even at pull date, vary markedly between manufacturers.

Other saprophytic and indicator organisms, including micrococci, presumptive group D streptococci, coliform bacteria and presumptive E. coli, did not represent an important part of the ham microflora and did not grow appreciably as a result of storage at 4 C. The types of group D streptococci on KF agar in this study were not determined. Even so, with presumptive E. coli counts <3/10 g it is unlikely that the group D streptococci indicate faecal contamination. However, in bologna (Paradis and Stiles, 1978b), these organisms grew during storage. This could be due to differences in composition between ham and bologna, or to the fact that idealized storage conditions created unfavourable conditions in these ham samples.

Potential pathogens, B. cereus, Salmonella and S. aureus were not detected at the minimum detectable level of 100/g for B. cereus and S. aureus, and was absent in 25 g in the case of Salmonella. Since meat products are a major vehicle of food-borne B. cereus poisoning (Goepfert et al., 1972), and S. aureus is cited as the real health hazard in

vacuum packaged meats (Hughes, 1970), the results of this study further confirm the apparent safety of the vacuum packaged luncheon meats analyzed in this laboratory (see Paradis and Stiles, 1978a; Stiles and Ng, 1979a). There was no indication that these products with total counts as high as 10^9 and 10^{10} per g were in any way unsafe for human consumption, and no overt signs of spoilage were apparent.

IV. Growth of Saprophytic Microflora and Inoculated Pathogens on Ham Slices in Sandwiches.

1. Abstract

Sandwiches made with freshly produced (new) and pull date (old) vacuum packaged, sliced ham were inoculated with a mixture of five enteropathogenic bacteria. Saprophytic microflora and inoculated pathogens were monitored. Lactic acid bacteria predominated the count of old samples, and presumptive group D streptococci and Microbacterium thermosphactum grew in new product, but did not predominate the population. Severely abusive storage (30 C for up to 24 h) of new ham inoculated and used in sandwiches, resulted in high counts of Staphylococcus aureus and Bacillus cereus, intermediate growth levels of Escherichia coli and Salmonella typhimurium, but no growth of Clostridium perfringens. No growth of enteropathogens occurred in old (high competition) product, but E. coli and S. typhimurium survived without significant decreases in their counts.

2. Introduction.

Meat is one of the principal foods involved in food poisoning outbreaks. However, mishandling and improper storage can generally be identified where outbreaks of food poisoning occur. Sandwiches are prepared almost entirely by hand, and contain a variety of perishable foods which are known to support the growth of bacteria (Khan and McCaskey, 1973; McCroan et al., 1964). Sandwiches are not generally

heated before consumption, hence the ability of enteropathogenic bacteria to grow becomes particularly significant. In their examination of prewrapped sandwiches, Hall et al. (1967) found that 16.7% of the samples had coagulase positive staphylococci, 9.2% contained C. perfringens, 3.7% had typical B. cereus strains, 34.2% had enterococci, and 16.3% had E. coli. No salmonellae were found in commercially prepared sandwiches (Hall et al., 1967; Khan and McCaskey, 1973; McCroan et al., 1964). Despite the potential for food poisoning from sandwiches, there are only a few reports of food poisoning outbreaks in which sandwiches have been implicated (Adame et al., 1960; McCroan et al., 1964; Todd, 1976).

Paradis and Stiles (1978b) studied the food poisoning potential of enteropathogenic bacteria inoculated onto bologna in sandwiches. They showed that severe temperature abuse was necessary to develop a food poisoning potential in the sandwiches. Ham was selected as the luncheon meat for this study. Vacuum packaged ham represents a relatively inhibitory environment, influenced by nitrite and salt concentrations, pH, partial oxygen and carbon dioxide pressures, storage temperature and competition. Besides cooking the ham during processing, slicing and vacuum packaging have a marked effect on microbial growth, and lactic acid bacteria are generally reported to predominate in vacuum packaged, cured meats (Allen and Foster, 1960; Alm et al., 1961; Cavett, 1962; Kempton and Bobier, 1970;

Lechowich, 1971 Mol et al., 1971; Reuter, 1973).

Curing, smoking and cooking of processed meats combine to select gram positive bacteria, including Micrococcus, Sarcina, Lactobacillus, Microbacterium and Bacillus spp. (Heiszler et al., 1972). As a result, both Staphylococcus aureus (Hughes, 1970), and Bacillus cereus (Graham and Blumer, 1971) could represent a hazard in cured meats

The objective of this study was to determine the food poisoning potential of enteropathogenic bacteria inoculated onto ham in sandwiches. In an earlier study (See Chapter 3) it was noted that ham from different manufacturers differed in microbial load and pH, not only with age, but also between manufacturers. Hence, product was selected for this study that would show the effects of microbial competition on the growth of food poisoning organisms in product from two different manufacturers.

3. Methods and Materials

A. Sampling.

Freshly produced, sliced but not vacuum packaged ham was obtained from two manufacturers, selected on the basis of a survey study (See Chapter 3). Product from manufacturer A was selected because of significantly lower counts and significantly higher pH than other manufacturers' product, whereas product from manufacturer B was selected because it had the greatest pH drop between new and old (pull date) product. The sliced ham was returned to the laboratory,

vacuum packaged in aluminum-nylon-polypropylene pouches (Cryovac Division, Grace Chemicals, Mississauga, Canada) under 26 lb/in^2 vacuum, with 10 slices per package. Packaged product was stored at 4 C for 24 h and 30 ± 2 days before being used in the inoculation studies.

B. Inoculation Studies.

Ham sandwiches were prepared using enriched white bread, spread with soft margarine (Kraft) and equilibrated at 21 C for 18 - 24 h. The ham packages were opened and inoculated and uninoculated (control) sandwiches were prepared. For control sandwiches, one slice of ham was placed between 2 slices of bread. The inoculated sandwiches were prepared by first spreading 0.05 ml of an inoculum mixture onto each ham slice before placing it between the two slices of bread. The inoculum mixture consisted of five enteropathogenic bacteria: Bacillus cereus B4AC, Clostridium perfringens 8239-H, Escherichia coli B7A, Salmonella typhimurium ATCC 13311, and Staphylococcus aureus S-6 (all except S. typhimurium were obtained from Dr. A. Hauschild, Health Protection Branch, Health and Welfare Canada, Ottawa). Cultures, except C. perfringens, were maintained on Tryptic Soy agar (Difco) and grown in Tryptic Soy broth (Difco) at 35 C for 24 h before use in the inoculum. C. perfringens was maintained and grown at 35 C for 24 h in freshly boiled, Cooked Meat medium (Difco). The mixture of enteropathogens was designed to give 10^2 - 10^3 of each

organism per slice. Actual inoculum concentrations were determined by enumerating appropriate dilutions of the inoculum mixture on the selective media. Prepared sandwiches were analysed for the enteropathogens within one hour of inoculating the ham slices (0 h sample), and held at 30, 21, and 4 C for 4, 8 and 24 h for further testing.

C. Bacteriology.

The saprophytic and pathogenic bacterial loads were determined using the entire ham slice from each sandwich. The slice was weighed aseptically, and blended with 99 ml of sterile, 0.1% peptone water in a Waring Blendor jar for 2 min at high speed. Appropriate dilutions were prepared in 99 ml sterile, 0.1% peptone water blanks and plated onto growth media. The media and incubation conditions are shown in Table 7. Confirmatory tests for enteropathogenic bacteria included: gram stain and catalase tests for B. cereus and C. perfringens; gas production at 45.5 ± 0.05 C for E. coli; growth on TSI slants and serological typing with Salmonella O antiserum Poly A-I and Group B factors 1, 4, 5, and 12 for S. typhimurium; and coagulase tests using EDTA coagulase plasma (Difco) according to prescribed methods (HPB, 1974). Confirmation of specific enteropathogens was also inferred from absence of typical test organisms on the control samples. In one instance, because of extensive growth of bile precipitating colonies on both control and test plates, a presumptive E. coli count on VRBA could not be obtained.

Table 7. Media and incubation procedures used to determine saprophytic, indicator and pathogenic counts on uninoculated and inoculated ham in sandwiches.

Count	Code	Media and Incubation
Standard Plate count	SP 35	Plate Count Agar (Difco), 35 C, 48 h.
Total Aerobic count	SP 21	Plate Count Agar (Difco), 21 C, 72 h.
Psychrotroph count	PSY	Plate Count Agar (Difco), 4 C, 10 days.
Presumptive Group D Streptococci	KF	KF Streptococcus Agar (Difco), 35 C, 24 and 48 h.
Lactic Acid Bacteria	APT	APT Agar (Difco), 30 C, 48 h.
Coliform and *E. coli* count	VRBA	Violet Red Bile Agar (Difco), 35 C, 24 and 48 h.
Presumptive *E. coli* count	LST	Lauryl Tryptose broth (Difco), 35 C, 24 and 48 h; and gas positive tubes into EC medium.
	EC	EC medium (Difco), 45.5±0.05 C, 24 and 48 h.
M. thermosphactum	STAA	Streptomycin Sulphate-Thallous Acetate-Actidione Agar 21 C, 48 h (Gardner, 1966).
B. cereus	MYP	Phenol red, Egg-yolk, Polymyxin Agar 30 C, 24 h. (Mossel *et al.*, 1967).
S. aureus	BP	Baird-Parker medium (according to Holbrook *et al.*, 1969). 35 C, 24 h.
C. perfringens	TSC	Tryptose-sulphite Cycloserine Agar (Difco), 35 C, 24 h; anaerobic.
S. typhimurium	BGA	Brilliant Green agar (Difco), 35 C, 24 h.

Hence, the enteropathogenic E. coli count was confirmed by the Most Probable Number (MPN) technique from EC medium (Thatcher and Clark, 1968).

D. pH and Moisture Determinations.

pH was determined on control (uninoculated) slices using a combination (single probe) electrode (Fisher Scientific Co., Cat. No. 13-639-90). Moisture was determined using an official method (AOAC, 1975).

4. Results

The microflora, pH and moisture contents of the samples were measured. The pH range for the 3 samples from each manufacturer, the means and the mean pH drop are shown in Table 8. Moisture contents of product from both manufacturers were almost identical (ca. 76%). Salt and nitrite levels, based on data supplied by the manufacturers, were 2% and 140 ppm (rate of addition), respectively. After cooking, residual nitrite ranged between 30 and 60 ppm. Nitrite levels in old (pull date) samples might be less due to depletion during storage (Sebranek et al., 1973). Other added ingredients were polyphosphate (0.3 - 0.4%), sodium ascorbate (0.04%) and sugar (ca. 1%).

Growth of the lactic acid bacteria is shown in Fig. 1. The count at 0 h for lactic acid bacteria on new ham from both manufacturers was 3.6 to 3.7 x 10^3/g. In the old (pull date) ham, lactic acid bacteria had increased to 4.9 x 10^8

Table 8. pH range and mean pH drop for new and old ham samples from manufacturers A and B.

Manufacturer	Mean pH value		pH range		Mean pH drop
	New	Old	New	Old	
A	6.26	5.88	6.20 -6.30	5.85 -5.90	0.38
B	6.33	5.81	6.20 -6.40	5.71 -5.88	0.52

and 1.2 x 10^9/g for product from manufacturers A and B, respectively. As a result, in new ham held at 30 C for up to 24 h there was a 10^5- to 10^6-fold increase in lactic acid bacteria. At 21 C in new ham this increase was 10- to 1,000-fold, compared to no change at 4 C for 24 h. In contrast, in old ham held under the same conditions, lactic acid bacteria counts showed little change when held 24 h, and any changes that did occur differed quite markedly between replicates.

Changes in the presumtive group D Streptococcus counts in new ham are shown in Fig. 2. Initial counts in new ham were 10 to 100/g. Increases of up to 10^4-fold in count occurred at 30 C, increases at 21 C were proportionately less, and at 4 C no significant change in count was observed within 24 h. In old ham (held 30 days at 4 C) presumptive group D Streptococcus counts exhibited widely varying counts at all incubation temperatures. However, subsequent holding, especially at 30 C for 24 h, resulted in a 100- to 1000-fold increase in count in product from manufacturer B and no increase in count in product from manufacturer A.

Another possible bacterium contributing to the total saprophytic count in vacuum packaged ham samples is M. thermosphactum. Initial M. thermosphactum counts in new ham samples were 10 to 1,000/g. In old ham stored at 4 C to pull date, M. thermosphactum counts had increased to 10^6 and 10^7/g for product from manufacturers A and B, respectively.

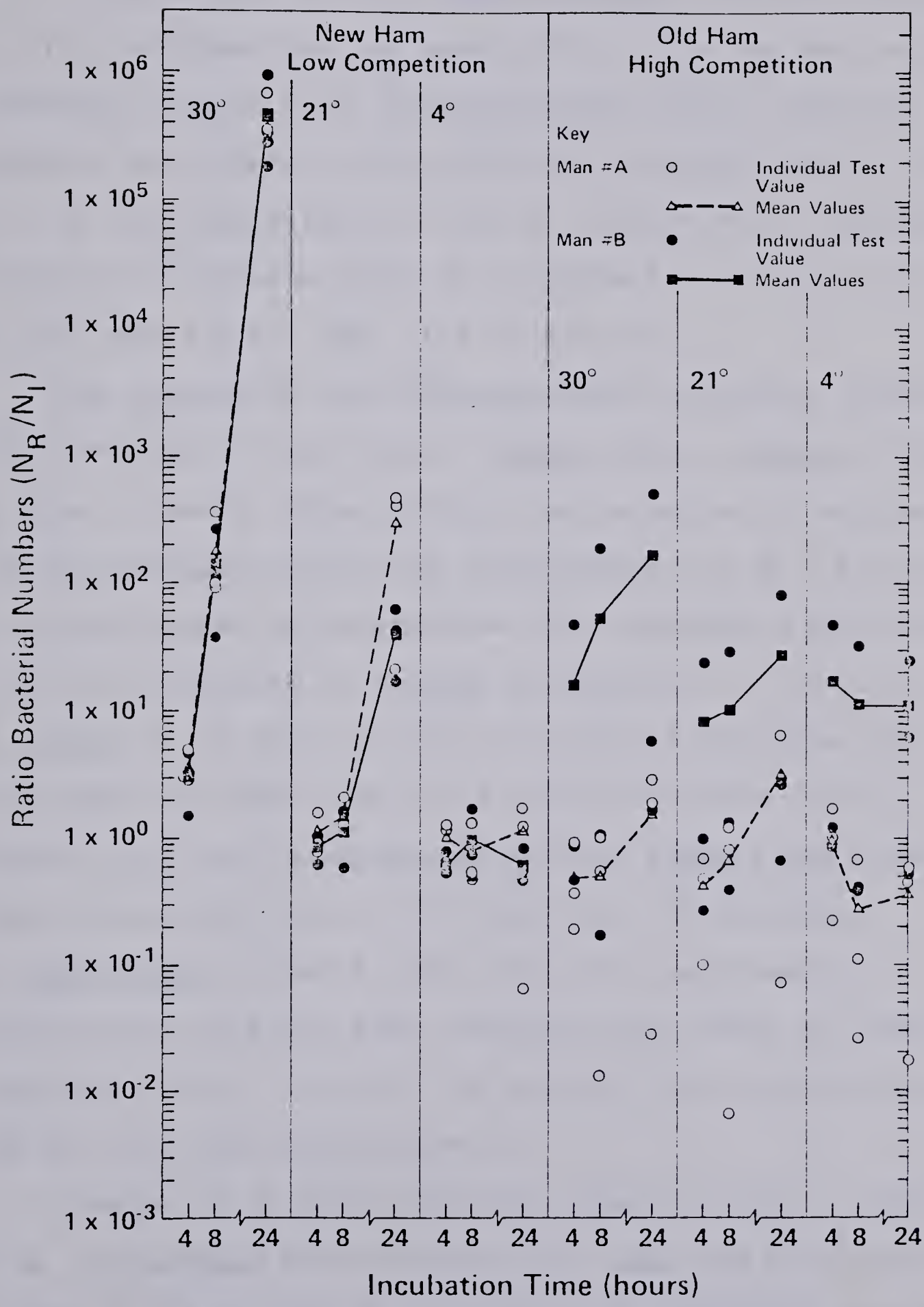

Figure 1. Change in lactic acid bacteria numbers with competition, and time and temperature of incubation.

However, at such levels, this organism only accounted for 1.0 and 2.8% of the respective total lactic acid bacteria populations. Under holding conditions of 30, 21 and 4 C for 24 h, further growth of M. thermosphactum was only recorded at 21 C, as shown for new ham in Fig. 3. In old ham, widely differing changes in M. thermosphactum counts occurred. However, the counts of 10^6 and 10^7/g (attained after 10 days at 4 C) for manufacturers A and B, respectively, exhibited a tendency to decrease after 24 h incubation at 30 and 21 C in all old ham (30 ± 2 days at 4 C) samples.

The changes in the enteropathogenic bacteria counts were recorded and data for B. cereus and S. aureus are shown in Figs. 4 and 5, respectively. The patterns are similar, and only holding of new ham in sandwiches at 30 C for up to 24 h represented an appreciable food poisoning hazard with these two pathogens. B. cereus increased up to 10^5-fold and S. aureus up to 10^6- or 10^7-fold. At 21 C increases for both organisms were much less, only up to 100-fold in 24 h. However, old ham in sandwiches did not support the growth of these organisms, even at 30 C for 24 h. In contrast, C. perfringens failed to grow under all experimental conditions, and there was a tendency for counts to decrease, especially at 30 C and 21 C on new ham from manufacturer B and old ham from manufacturer A.

Changes in E. coli counts are shown in Fig. 6. Changes in S. typhimurium were similar to E. coli and are presented in Fig. 7. In new ham at 30 C for 24 h, increases were about

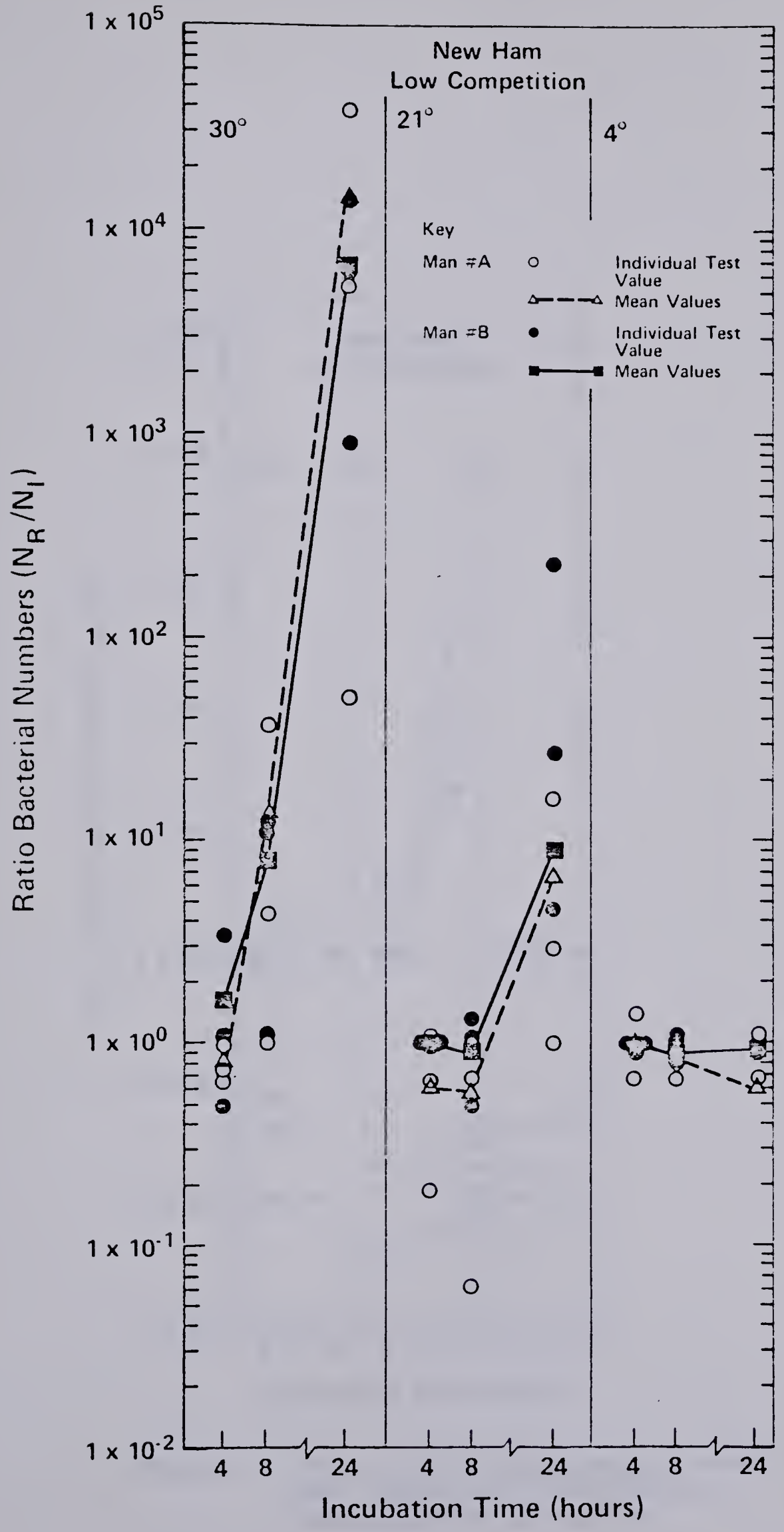

Figure 2. Change in presumptive Streptococcus numbers with competition, and time and temperature of incubation.

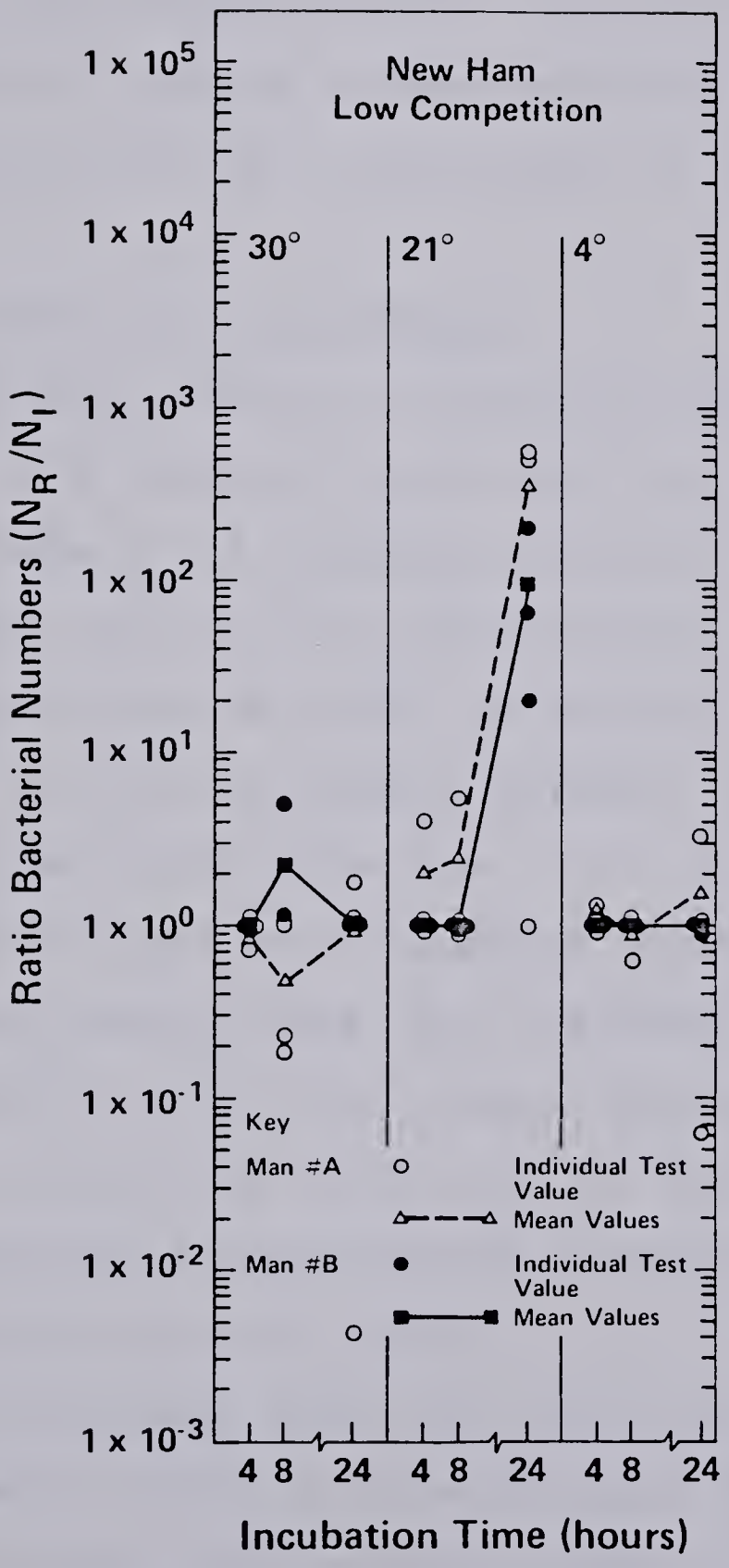

Figure 3. Change in M. thermosphactum numbers with competition, and time and temperature of incubation.

1,000-fold, and only 10 to 100-fold at 21 C within 24 h. In old ham, the changes in E. coli and S. typhimurium varied. No increases were observed in E. coli numbers on old product from both manufacturers. No changes in S. typhimurium were observed on old product from manufacturer A, but on old product from manufacturer B, the change in S. typhimurium numbers was variable between replicates, with increases up to 1,000-fold at 30 C and 100-fold at 21 C.

5. Discussion and Conclusions

The market survey of vacuum packaged, sliced ham samples (see Chapter 3) indicated that product from manufacturer A had significantly lower saprophytic counts and higher pH than other manufacturers' product. Product from manufacturer B showed the greatest pH drop between new and old (pull date) product. However, in this inoculation study, these differences were not as apparent, hence marked differences might not be expected between the product of these two manufacturers. This contrasted markedly with pH differences observed for bologna (Paradis and Stiles, 1978b) and for chopped ham (Stiles and Ng, 1979a). In the latter study, chopped ham product was obtained from the same manufacturers as this study.

In the survey study, the total counts at 35, 21 and 4 C were similar to the count on APT agar. APT is widely used to indicate lactic acid bacteria counts where these organisms are reported to predominate (Kempton and Bobier, 1970).

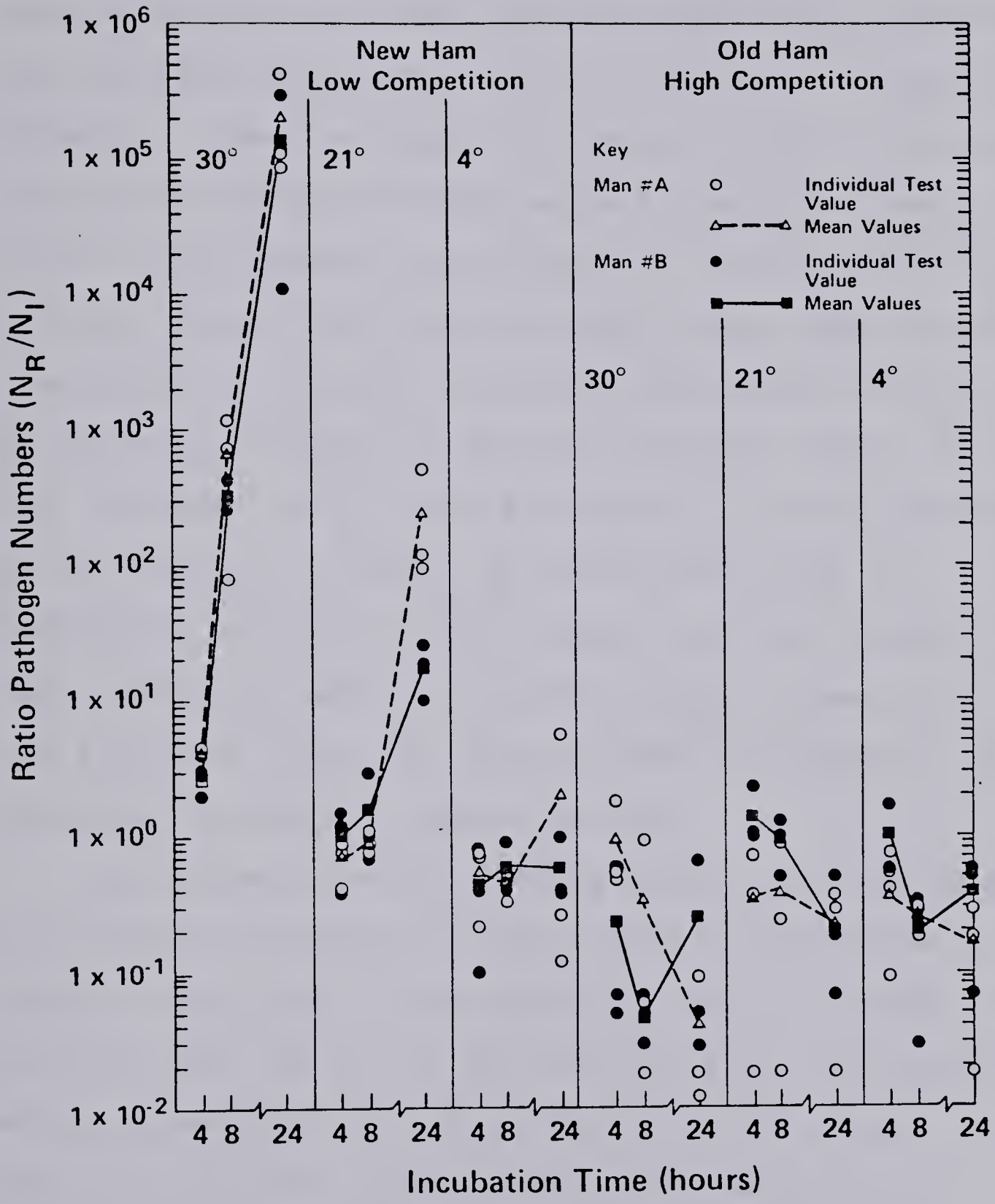

Figure 4. Change in B. cereus numbers with competition, and time and temperature of incubation.

Although APT is not selective for lactic acid bacteria (Kitchell and Shaw, 1973), Kempton and Bobier (1970) indicated that these organisms rapidly outgrew all others. Based on reports on vacuum packaged meats which indicated that the total saprophytic count is primarily lactic acid bacteria, a lower pH might be expected in ham in which the total count had achieved the maximum population levels of 10^8 to 10^9/g. However, the relatively limited pH drop of 0.4 to 0.5 pH units, might be attributed to the lower content of fermentable carbohydrate (Kempton and Bobier, 1970), or to the buffering capacity of the meat (Frazier, 1967; Ingram, 1962; Pearson, 1971). Since the pH drop in vacuum packaged ham is limited to <1 unit, pH is unlikely to be a significant protective factor against pathogen growth in these products, unless this limited drop, in association with the other inhibitory factors cited in Chapter 2, is sufficient to inhibit pathogen growth.

The apparent loss of viabilty of the enteropathogens when inoculated onto ham slices, could be attributed to injury or death due to environmental changes. However, it indicates that low levels of pathogens might be difficult to detect, especially with the limits of most plating techniques in which minimum detectable counts are <3 organisms/10g (*E. coli*) and 100 organisms/g (*C. perfringens* *B. cereus*, *S. aureus* and *S. typhimurium*). With severely abusive conditions such as 30 C holding for up to 24 h, both *B. cereus* and *S. aureus* could grow from virtually

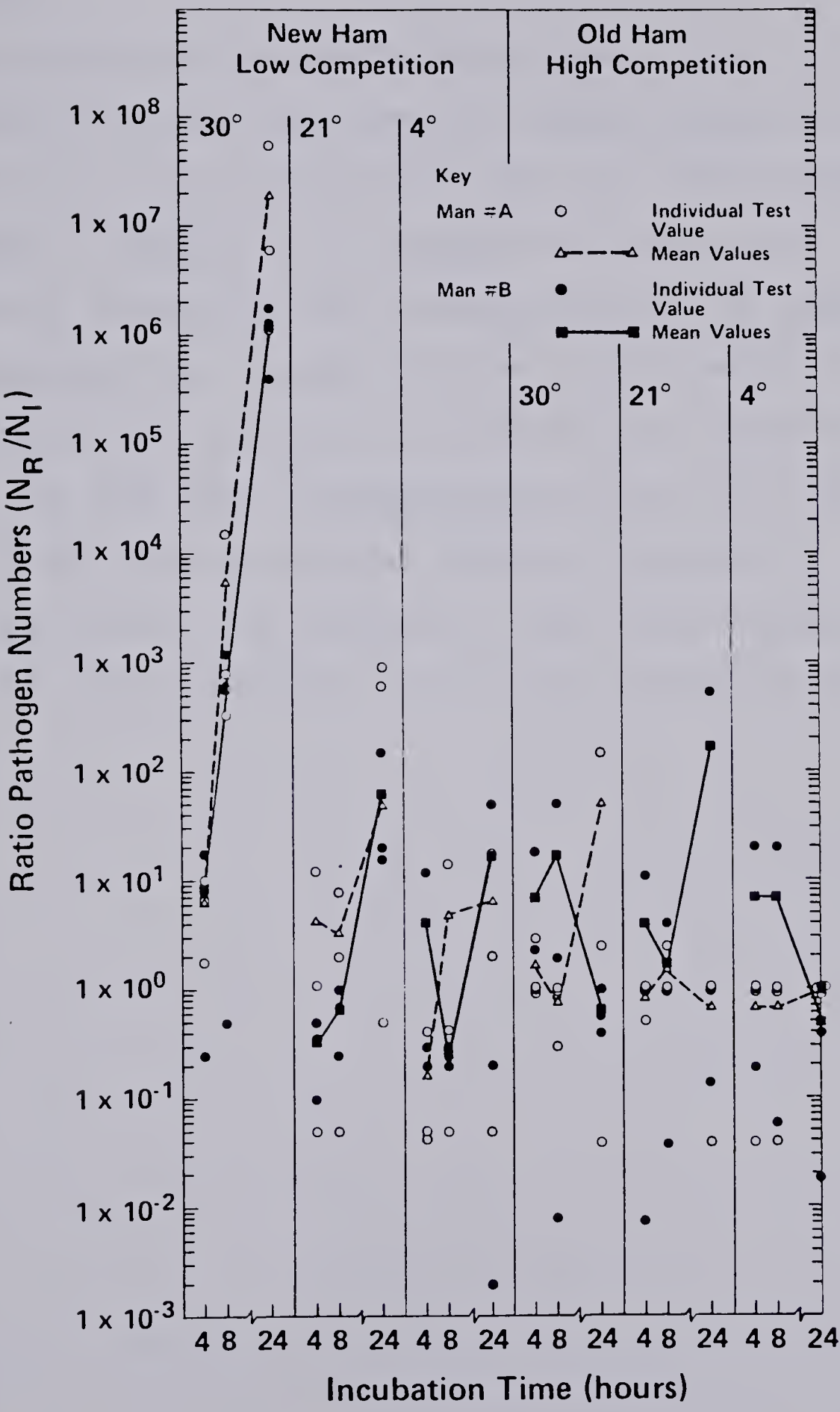

Figure 5. Change in S. aureus numbers with competition, and time and temperature of incubation.

undetectable levels in normal enumeration tests to potentially hazardous levels that could cause food poisoning.

The results for enteropathogen growth on ham in sandwiches indicated that severely abusive conditions of 30 C for up to 24 h was necessary for the enterotoxigenic pathogens, B. cereus and S. aureus, to develop to a potentially hazardous level. Enteropathogenic E. coli and S. typhimurium were capable of growth under severely abusive conditions in the presence of low levels of competitors. However, E. coli and S. typhimurium survived, but did not grow, in the presence of high counts of competitive organisms (lactic acid bacteria). Since these organisms are infective, their survival in ham is as significant as their growth.

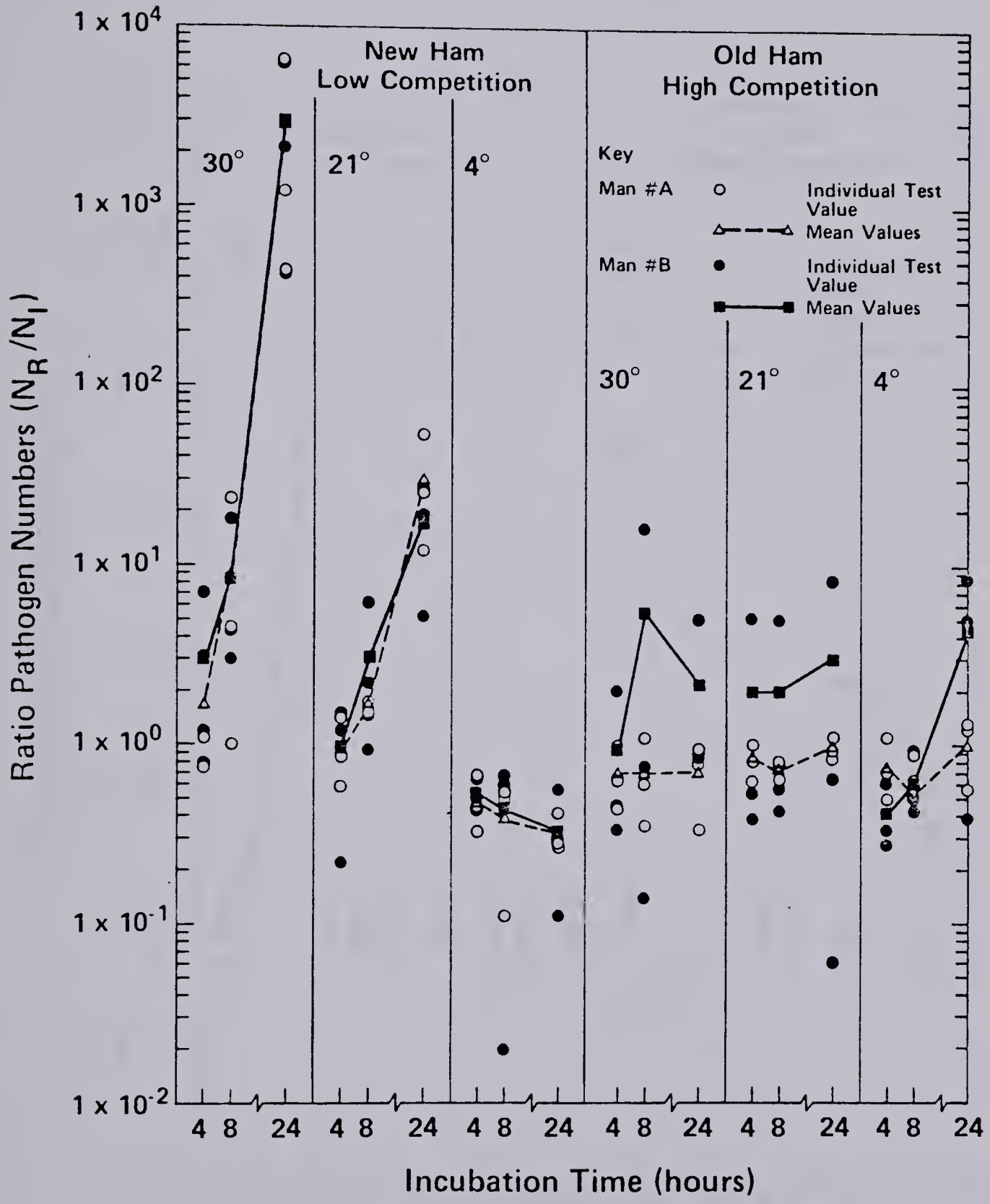

Figure 6. Change in E. coli numbers with competition, and time and temperature of incubation.

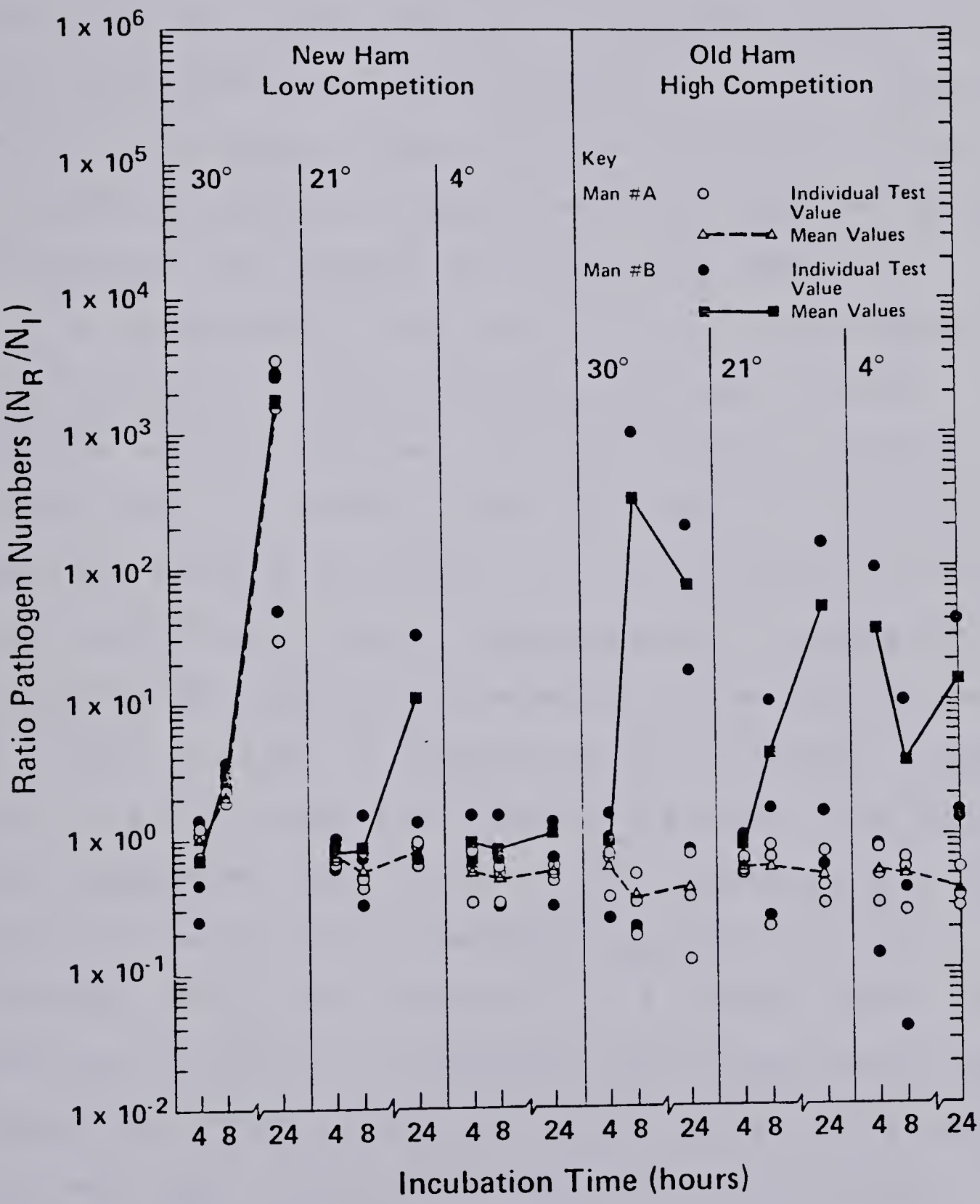

Figure 7. Change in S. typhimurium numbers with competition, and time and temperature of incubation.

V. Summary and Conclusions.

This study completes a series which investigated the microbiology of vacuum packaged, sliced luncheon meats, and the food poisoning potential of enteropathogenic bacteria inoculated onto these products in sandwiches. Three meat types were tested: an integral meat, ham, in this study; an emulsion type meat, bologna (Paradis and Stiles, 1978b); and a combination product, chopped ham, which combines integral and emulsion type product (Stiles and Ng, 1979a, b).

An increasingly large amount of cured meat products are being marketed in vacuum packages, and many of these products support the growth of food poisoning organisms (Christiansen and Foster, 1965). The anaerobic packaging of foods may increase the hazard of food poisoning by allowing toxin production by both C. botulinum and S. aureus while minimizing the activities of spoilage organisms (Thatcher et al., 1962). In fact, S. aureus have been reported to grow well and produce toxins on cured meat products with reduced (non competitive) flora (Anon., 1973; Riemann et al., 1972). Preformed toxins are not destroyed by acid conditions (Dempster, 1976), and lowering of pH to levels needed for effective inhibition of vegetative cells takes several weeks (Kempton and Bobier, 1970). As a result, acid conditions have been reported to provide only partial inhibition, especially in the case of S. aureus (Dempster, 1976). Since enteropathogens like Salmonella and E. coli are not toxigenic, it is likely that low pH could be an effective

inhibitor.

Although C. botulinum (Thatcher et al., 1962), and B. cereus (Goepfert et al., 1972) have been cited as potentially hazardous organisms, cured meat products have only rarely been associated with food poisoning outbreaks caused by these enteropathogens (Goepfert et al., 1972; Insalata et al., 1969). S. aureus is considered the real hazard in cured meat products (Hughes, 1970), and there have been reports of this organism being involved in food-borne incidents . C. perfringens food poisoning outbreaks remain common (Thatcher, 1963), but semi-preserved meats have seldom been implicated (Riemann et al., 1972; Todd, 1976). Salmonellae and E. coli are readily destroyed by the heat processing applied to these products (Smith et al., 1975; Surkiewicz et al., 1977). As such, the public health concern with respect to cured meat products focuses on the spore formers and the salt tolerant, heat resistant microorganisms. However, reports in the literature indicate that the commercial levels of nitrite and salt coupled with low pH can inhibit growth of the vegetative cells of these pathogens, as well as spore germination and outgrowth in the case of spore formers.

The results from the survey study indicated that vacuum packaged ham is predominated by lactic acid bacteria, and that potential pathogens, including B. cereus, C. perfringens, E. coli, S. aureus, and salmonellae, could not be detected at their respective minimum detectable

levels. Hence, it may be concluded from the results of the survey study that this product is apparently safe. Total counts of 10^7 and 10^8/g were attained on new ham (<10 days from manufacture). On old ham (stored to pull date at 4 C), total counts reached levels of 10^9 to 10^{10}/g. However, there is no indication that the old product, with such high total counts, is in any way unsafe for human consumption. In addition, no overt signs of spoilage, even in old ham, were apparent.

Sandwiches are usually prepared from a variety of perishable foods which could support growth of bacteria (Khan and McCaskey, 1973; McCroan et al., 1964). In addition, because of handling and storage abuse, growth of enteropathogenic bacteria is a real possibility (Longree et al., 1959; McCroan et al., 1964). However, sandwiches have rarely been implicated in food poisoning incidents (Adame et al., 1960; Todd, 1976), and only in cases of severe abuse were they able to develop a food poisoning potential (Paradis and Stiles, 1978b).

The fate of the 5 enteropathogens on ham in sandwiches was similar to the two other products tested. In addition, the fate of these enteropathogens was similar whether conditions were designed to simulate consumer contamination (Paradis and Stiles, 1978b; Stiles and Ng, 1979a) or manufacturer contamination (Stiles and Ng, 1979b). In all cases severe abuse was necessary to produce a food poisoning potential with enterotoxigenic bacteria, B. cereus and

S. aureus. However, in cases of severe abuse, counts of B. cereus and S. aureus below their minimum detectable level represent a potential hazard. In contrast, C. perfringens was not a potential food poisoning hazard under any of the experimental conditions. The gram negative, infective enteropathogens, E. coli and S. typhimurium, grew partially under extremely abusive conditions in low to moderate competition product. However, they survived under almost all experimental conditions indicating a food poisoning potential if these products are contaminated.

To conclude, these studies indicated that ham, chopped ham or bologna as vacuum packaged, sliced luncheon meats are not particularly hazardous products. Furthermore, high microbial counts, which generally appear to be lactic acid bacteria, are more a safety factor in their prevention of enteropathogen growth, than a hazard to the consumer.

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